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CONTENTS

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CONGENITAL ABSENCE OF LIMBS IN TORTOISES OF THE GENERA TRIONYX AND EMYDA

BY

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The justification for writing this note has arisen from the fact that occasional absence of limb in the Gangetic tortoises was observed in more than one specimen. Two specimens of the genera Trionyx and Emyda shewing such deformity were obtained during the course of the year. The one, Trionyx gangeticus Cuv measured 15″ × 12″ and lacked the right hind leg, while the other, Emyda granosa Schœpff measured 6″ × 4″ and was devoid of the right fore-limb. At the spot where the limb in question should have existed there was a dimple in the first and a fold of skin in the second tortoise. The two specimens were entirely normal in every other respect. In no case, however, was there any hint of a scar, thus *shewing that the absence of* the leg may be congenital instead of being due to an accident.

A deviation from the normal growth of the paired limb occurs now and again throughout the Vertebrate series, as the following brief account will show :

Fishes—In fishes, Hora, 1921 (11), has described occasional absence of paired fins in a number of genera A specimen of *Barilius barila* is described in which both the ventral fins were absent In another species *B dogarsinghi* the ventral fin of the left side was absent In *Nemachilus Lanjupkhulensis* the ventral fin of the right side was wanting and the other was abnormal In *Rita rita* the right pectoral fin was lacking, the musculature was degenerated and the shoulder girdle was abnormal In all these specimens of fish the limb-abnormalities were congenital defects as there was no trace of injury. Hora interprets them as mutations, or as due to some arrest in the growth of the organ

Willey, 1920 (21), has referred to the absence of the ventral fins in a male Amia clava

Jean Delphy, 1918 (4), describes cases of anomalous pelvic fins in *Cottus babulus* In one there seemed only one fin perhaps due to coalescence of both the right and the left fins. In another there was a reduction of one fin almost to a vanishing point perhaps due to some arrest of development at an early stage

Eigenmann and Cox, 1901 (9) and Brindlay, 1891 (2), have given description of abnormal fins in fishes

Amphibians—In the Urodele amphibian *Amblystoma punctatum* Harrison, 1921 (10), has shown experimentally that duplex and multiplex appendages frequently arise from the transplanted limb buds

Banta and Gartner, 1914 (1), have published the results of some observations on accessory appendages and other abnormalities due to the action of centrifugal force on amphibian larva There was usually only one accessory appendage to each animal though as many as four were noted The appendages were usually lateral or dorsolateral in position They were tail-like in external appearance

Abnormalities in hind limbs of *Rana* are described, and experimentally probable variability of the organ reported by several notable workers. Reichenow, 1908 (16), reports on a number of abnormalities in hind limbs of young frogs *Rana esculenta* One had only one hind leg, another three, and a third had four Woodland (22) has furnished descriptions of some similar cases *R tigrina* in which a stalk bearing a pair of additional limb is attached to the thigh of the left leg The aborted fused thighs of the additional pair of legs are represented by a small plate.

Harold Row, 1916 (17), describes a case of symmetry abnormal feet in *R temporaria* which showed an absence of the first digit. There is no trace of mutilation

Durkin, 1910 (6), has made experimental study of limbs in frog by extirpating the primordia of the limbs at a very early stage in the development of the animal The amputation of one limb rudiment is usually associated with serious malformation in others. He explains the phenomenon as due to a very interesting effect on the development of the central nervous system brought on by the extirpation of the limb bud there being a developmental correlation between the nervous system and the peripheral organs. The affected nervous system exerts an influence on the other limbs causing a defective growth.

Lissitzky, 1910 (13), has induced duplicity by cutting the primordia of limbs in young tadpole

Reptiles —Dnerden, 1922 (5), has observed in the South African lizard of the genus *Chamæsaura* that the three species show different degree of limb reduction. In *C. ænea* both pair of limbs are present but much reduced, in *C. anguina* both pairs are styliform and barely divided into two minutely clawed digits, in *C. macrolepis* the fore-limbs are absent and the two hind limbs are styliform and undivided. He does not, however, think that the three species form a series showing stages in the direction of

further reduction of limbs but is of opinion that the anlage or the germinal factor concerned with the limb production is or has been in a highly mutative state

Birds.– Zankewitsch, 1922 (29), has described abnormality in a Duck's wing A wing of *Anas boschas* showed on the ventral side in the region of carpo-metacarpus a hint of supernumerary limb The feathering of the supernumerary part, which also bore two claws, inclined to be wing-like

In a recent paper Roy, 1931 (18), has described heteromorphosis of the pelvic girdle, the presence of a pair of supernumerary hind legs and duplicity of cloacal openings in a domestic fowl. The pair of additional appendages were attached to the pygostyle by a peculiar conelike modification of the fused femur bones.

In another paper the writer in collaboration with Roy, 1931 (8), has given an account of the arrest in development of the right hind leg in a hen-feathered cock. The right femoral bone is represented as a small nodule attached to the acetabulum by the ligamentum teres.

Florence Peeble, 1910 (14), has made operation on the limb buds of chick The results indicate "that when the tip of a young bud is grafted on the proximal portion of another limb it becomes a part of the appendage to which it is attached instead of retaining the character of the part it is destined to become. No regeneration of the limbs takes place after the removal of the buds."

Mammals —An interesting account of congenital absence of both hind legs in an adult pig is given by Sumulong, 1926 (20). Carreon, 1919 (3), has reported absence of hind legs below femur in a full term pig. He explains the abnormality as due to some physico-chemical interference very early in the development of the pig.

Khurkham and Haggard, 1916 (12), described the structure of a three-legged Kitten—the left fore-limb being

apparently absent. The limb-hud, he tbinks, had encountered some obstaclo and checked its growth.

It will be seen from the above tbat reports on a number of nbnormalities of the appendages in Vertebrates have been contributed by good many observers, bnt no account seems to be extant in so far as the limb-abnormalities of the Chelonia are concerned The writer therefore takes the opportunity of describing the nuomalipeds

The photograph (Fig 1) shows the ventral aspect of the larger tortoise (Trionyx gangeticus Cuv) without the right bind leg The plates of tho plastron are perfectly normal and so nre those of the carapace. The plastral callosities aro large and normal. Tho visceral organs lie in proper situation without nny trnce of abnormnlity except tho right leg, wlnch is uppnrently absent. Tho musoles aro extremely degenerate in the particular region where the right leg should hnve been The right acetnbulum is very imperfectly got np and convex instead of concave, n structure suggestive of developmental arrest of tho leg and subsequent fusion with tho girdle The rest of the pelvic girdle is free from deformity. The blood vessels nnd the nervos are on a reduced scale only supplying the degenerate mnscles and connective tissue of the right leg

The younger specimen Emydn granosa Schœpff (Figs. 2 and 3) shows outwardly no indication of the existence of the right fore leg. The skin covering the spot is folded into wrinkles nnd on dissecting out tbe nnimal it was found that tho muscles nnd bones of this limb showed great retrogression in development. The bony part being redneed to a small nodule lay deeply imbedded in the flesh. This piece of bone measured 0·5″ × 0·3″ nnd wns nttached to the right glenoid of the pectoral girdle by a ligament. The girdle is well-formed nnd nornnl.

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The probable cause of the limb abnormality is due to some injury to the anlage or the germinal factor concerned

with the production of the limb at a very early stage of development. Stockard, 1921 (17), Patterson, 1907 (13) and several other observers have found the developmental interruption of the organ to exist in many animals in connection with other abnormalities besides the limbs At a very early stage of the development the central nervous system plays a very important rôle in co-ordinating the developmeot of limbs and other organs, and any disturbance to the central nervous system must necessarily cause serious defects in the body-building Reciprocally, as Durkin has showo, that hinderiog of the development of an orgao is followed by abnormal development in the whole central nervous system and from the affected nervous system an influence is exerted on other parts of the body causing deformities The disturbance may be brought about by inadequate supply of oxygen, food, or it may be caused by external shock or injury. According to Banta and Gartner, "the hereditary determinars for development work out their destined end only whao maintained in certain appropriate relation."

In the end the writer wishes to express his sincere thanks to Professor D. R. Bhattacharya for help and criticisms. The work was carried out under his direction in the Department of Zoology of the University of Allahabad

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Plate 1

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Plate 1

Plate 2

THE TRANSFERENCE OF GOLGI BODIES FROM THE FOLLICULAR EPITHELIUM TO THE EGG IN CERTAIN INDIAN SNAKES

BY

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INTRODUCTION

My work on the oogenesis of certain Indian snakes revealed many points of interest, not the least interesting of which has been the phenomenon of the transference of Golgi bodies from the theca and the follicular epithelium cells to the egg. The well-developed Ophidian oocyte has a fairly thick layer of peripheral Golgi bodies · such a condition, however, is not novel and is easily demonstrated in the earlier and later stages of most vertebrate and at least some invertebrate eggs The presence of a second type of Golgi bodies in the follicle cells and in the outermost confines of a fowl's egg led Brambell in 1924 to express the view that a good portion of them is derived from the follicular epithelial cells as a result of an inward migration—simultaneously with Bhattacharya who observed a similar phenomenon in tortoises Since then the latter author and his pupils, of whom the present writer is one, have examined a large number of vertebrate eggs and reported the infiltration of Golgi bodies, as they have called it, in tortoises, lizards, birds, fishes and frogs. The aim of the

present paper is to add to the types in which the infiltration of Golgi bodies from the theca and the follicular epithelium cells to the egg has been observed, and to indicate, so far as possible, the manner and the significance of the process

It is my pleasant duty to record my obligation to Dr D R Bhattacharya, Professor of Zoology in this University, to whose original idea of Golgi infiltration the present paper owes its origin, for his unfailing courtesy in help and guidance.

MATERIAL AND TECHNIQUE

Ovaries were taken and fixed from the following five types of snakes—Zamenis mucosus, Eryx conicus, Tropidonotus stolatus, Tropidonotus piscator, Gongylophis conicus All possible precautions as recommended by Gatenby and Cowdry (8) during the period of transference of the ovary from the body of the animal to the fixatives were taken Of the large number of fixatives which were employed the following gave good results in showing clearly the egg membranes and demonstrating the Golgi bodies in various structures

(1) DaFano's Cobalt-Nitrate Method

(2) Cajal's Uranium-Nitrate Method.

(3) Ludford's latest modification of the Osmic Acid Method

(4) Flemming without Acetic Acid.

In the case of (1) and (2), the sections were toned with 1 per cent Gold chloride, 5 per cent Hypo and 3 per cent Ammonium sulphocyanide. The osmiophilic intensity of a snake oocyte being much less than is the case in tortoises and other vertebrate eggs, it was not found necessary to bleach the eggs by Henneguy's method

EGG MEMBRANES

The earliest oocytes of the snakes examined are lodged in investments of thecal tissue, which unlike their anlage in pigeon develop as a single layer and are not divided into Theca interna and Theca externa As the oocyte grows, the follicular epithelium is formed as a multi-layered structure and this condition is assumed at an early stage of development The individual cells are at first small but soon grow into what is usually a middle layer of large oval cells having a nucleus and a prominent nucleolus, and a few layers of smaller cells on the inner and outer sides The cells next to the zona radiata are specially small and often crowded together.

In between the follicle cells and the egg, the only structure visible is a narrow faintly striated layer which usually does not take much stain and is not quite transparent. This, without doubt, correspondo to the zona radiata so well developed in tortoises and visible as a single layer in lizards and birds There is, however, no trace of any fibrillæ layer found in tortoises, nor could any well-formed 'limiting membrane' as noticed in other reptiles and birds, be distinctly made out. The zona radiata usually develops at a late stage of oocyte growth.

GOLGI MIGRATION

In well-developed oocytes of Eryx conicus and Tropidonotus stolatus, Golgi bodies are present in the theca and in the follicular epithelium in large numbers (Figs. 1 and 2) In Lndford and F.W.A preparations they appear as dark granules or as crescents. Often many of the granules are aggregated together, exactly in the same manner as they do in the extreme peripheral region of the egg The actual transference of the Golgi bodies from these layers takes place in

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bodies and appear very dark in a DaFano preparation. No definite method of transference can be made out here, for there seems to be none, but the fact of the transference itself is too apparent A similar stage of Zamenis mucosus shows a many-layered follicular epithelium of small cells, the zona radiata unformed, but the activity of infiltration equally intense.

DISCUSSION

The condition of the egg membranes is almost similar to that described by Mlle. Loyez in 'Ophidians' where (1) the follicular epithelium is said to contain 'small cells which can divide mitotically, (2) intermediate cells arise from the differentiation of the small cells in the inner layer of the follicle, (3) large pear-shaped cells result from the development of the intermediate elements' The large pear-shaped cells are, however, well-marked features of the follicular epithelium of well advanced oocytes other smaller cells are present in all stages of development and their division into smaller and intermediate cells in the form of definite layers can hardly be justified.

The behaviour of the Golgi bodies with respect to their migration is almost similar in all the five types examined In all of them infiltration becomes active and marked in certain stages in the development of the oocyte, and the onset of the phenomenon is noticed specially in the later stages An early oocyte even with a well-formed follicular epithelium shows hardly any Golgi bodies in the latter structure, much less any signs of infiltration This seems to be directly in contrast with the condition in fowls where according to Brambell (6) "the process of intrusion of elements from the follicle ceases at the time when the one-layered follicle becomes many-layered and commences to secrete zona striata."

isolated units, each individual granule working its way only inwards No chains of migrating Golgi bodies or regular passages, such as has been reported in tortoises by Bhattacharya (1), or little lumps as was first indicated in fowl by Brambell (6), or as described in lizards and in birds by Datta and Das (3) respectively, could be observed in any of the five Ophidian types examined

In a well grown oocyte of Zamenis mucosus, Golgi bodies are present in fairly large numbers in the theca as well as in the follicular epithelium cells In the latter the dictyosomes often aggregate closely around the nucleus on the side nearest to the zona radiata Thence they seem to be continually migrating, in a measured degree as it were, to the outer layer and finally to the cortical region of the egg These migrating Golgi bodies are commonly seen almost at all places in a well preserved zona radiata. A very clear instance of their crossing the last limits of the zona radiata and almost stepping into the confines of the egg is shown in Fig 5, which is a Cajal preparation of Gongylophis oocyte Probably by mere coincidence or as a result of rotation the Golgi bodies have come on the innermost area of the zona radiata simultaneously throughout the circumference of the egg and are caught in the act of entering it

Figure 4 is an earlier stage of the oocyte of Tropidonotus piscator. Only one definite layer of follicular epithelium cells is prominently seen A few smaller cells perhaps are also present and the boundaries of the individual cells are not very well defined The zona radiata has not yet developed The number of Golgi bodies present in the follicle cells is immense and their passage to the periphery of the egg wholesale, so much so that there is hardly any dividing line visible between the oocyte and the follicular epithelium The cortical region of the egg as well as the follicular cells are thick with Golgi

bodies and appear very dark in a DaFano preparation. No definite method of transference can be made out here, for there seems to be none, but the fact of the transference itself is too apparent A similar stage of Zamenis mucosus shows a many-layered follicular epithelium of small cells, the zona radiata unformed, but the activity of infiltration equally intense

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The Golgi bodies that pass out from the follicle cells are fairly large irregular bodies and not unoften two or three elements are inextricably fused together. This is specially the case in Eryx conicus and Tropidonotus stolatus where the tendency to aggregate in clusters is most marked. It is, therefore, obvious that a zona radiata with definite channels and a fibrillar layer with similar canaliculi should be more a hindrance than help for the passage inwards of these bodies, transmission through which is only possible for dust-like particles.

Unlike the case in fowls the appearance of the zona radiata does not seem either to commence or to stop the infiltration activity of the follicle cells. Only in the absence of this layer the transmission of the Golgi body is more prolific and haphazard. Miss Thing (9) has stated that "the structure of the zona pellucida," and this term includes zona radiata, "presents a condition most favourable for the conveyance of nutritive material from the epithelial area in contact with the maternal capillaries to the actively growing and extending yolk." Considering the part played by the zona radiata in the oocytes of snakes, in this connection, it seems possible to suggest that this layer acts not only as a vehicle of Golgi transmission in virtue of the incidence of its position, but also as an active medium regulating the inflow of the Golgi bodies which would otherwise filter down in utter disregard of the metabolic needs of the growing egg. For after all as Waldeyer, Mlle Loyez and others have pointed out the transmission of the Golgi bodies from outside to the egg must have a definite role to play in the economy of the latter body and it is natural to suppose that this process should have greater chances of success when the inflow is measured and regulated than otherwise.

The infiltrating Golgi bodies have nothing to distinguish them either from those elements that are in the follicle cells or from those found in the oocyte.

They settle down, as in other animals, in the cortical region which becomes dark and thick being packed full of them, particularly in DaFaun and Cajal preparations. This is specially the case prior to the formation of the zona radiata After the emergence of this layer the Golgi bodies continue to swell, aggregate or even fragment in the cortical layer and are gradually used up.

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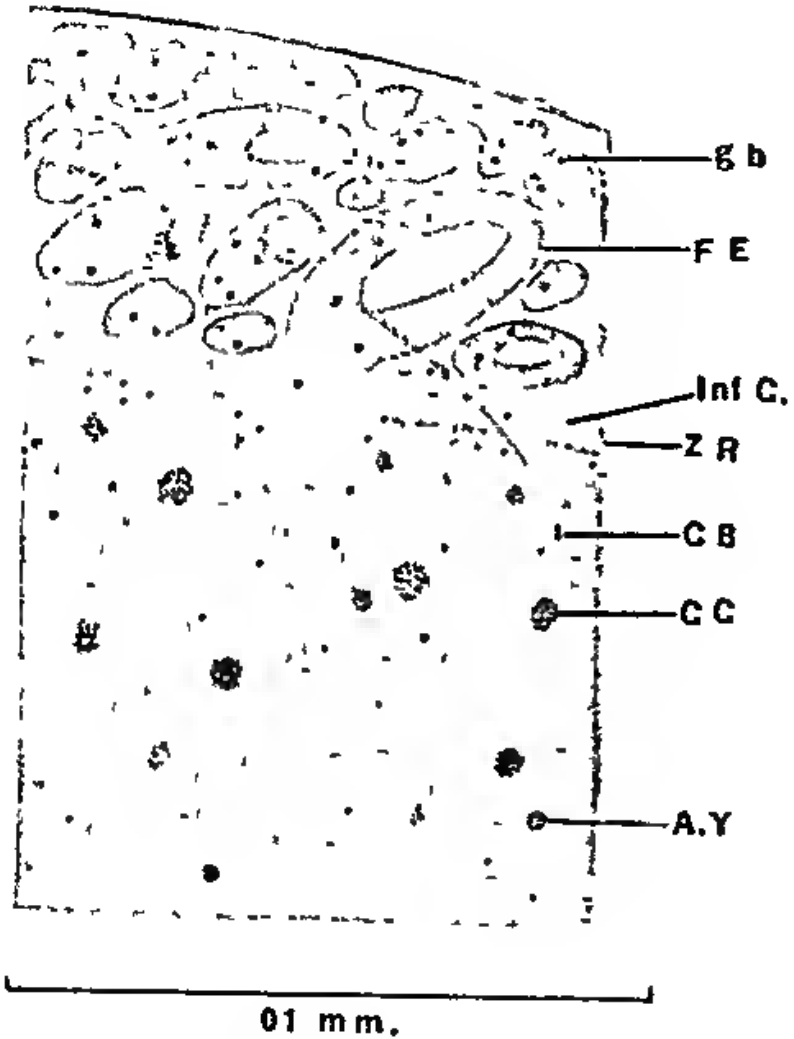

Fig 1

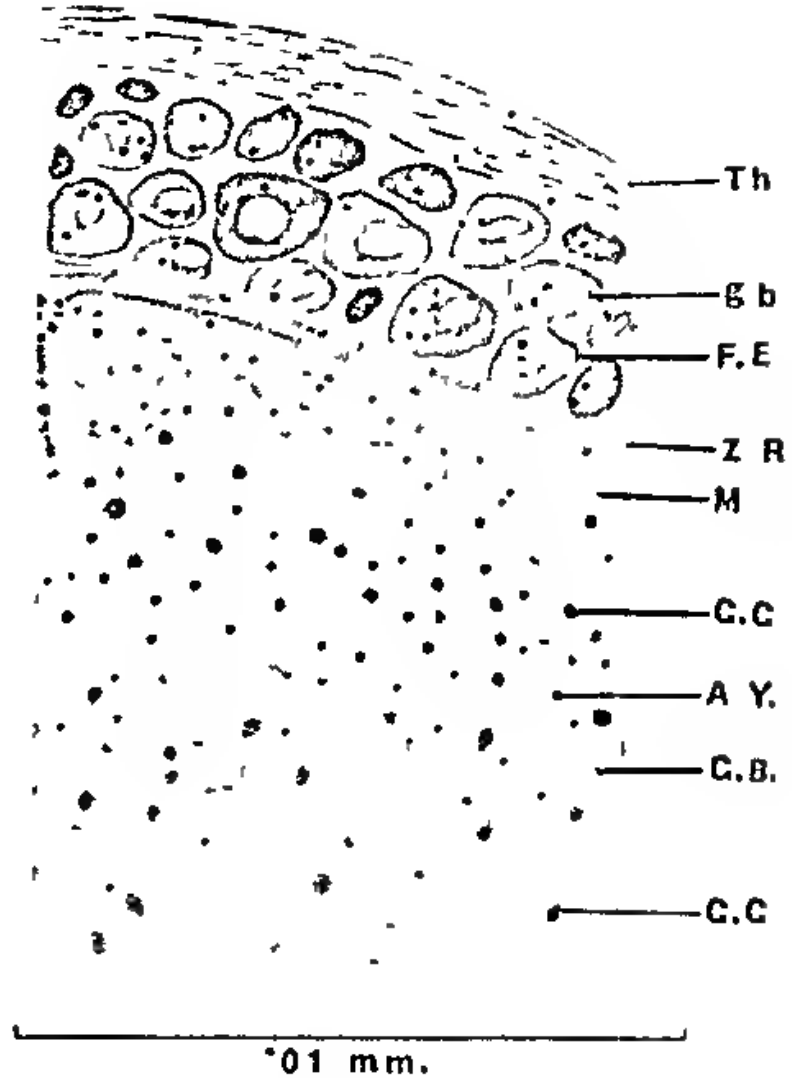

Fig 2

Plate 1

of Golgi elements Fairly advanced egg of Tropidonotus stolatus in which albuminous yolk is also developed F.W.A fixation Champy-Kull stain

Fig 3 —Large pear-shaped follicular cells with smaller ones on the sides A distinct zona radiata Infiltrating activity slow and measured Full-grown egg of Zamenis mucosus Ludford's latest osmic fixation

Fig 4 —Follicular epithelium hardly more than one-layered. No trace of zona radiata Infiltration haphazard and excessive Tropidonotus piscator oocyte Dal'ano fixation Saffranin and Light Green stain

Fig 5—Infiltrating activity at its highest though the presence of zona radiata seems to regulate the process Large pear-shaped cells in the central follicular layer. An advanced egg of Gongylophis conicus Cajal fixation Toned with Gold chloride and Hypo

Plate 2

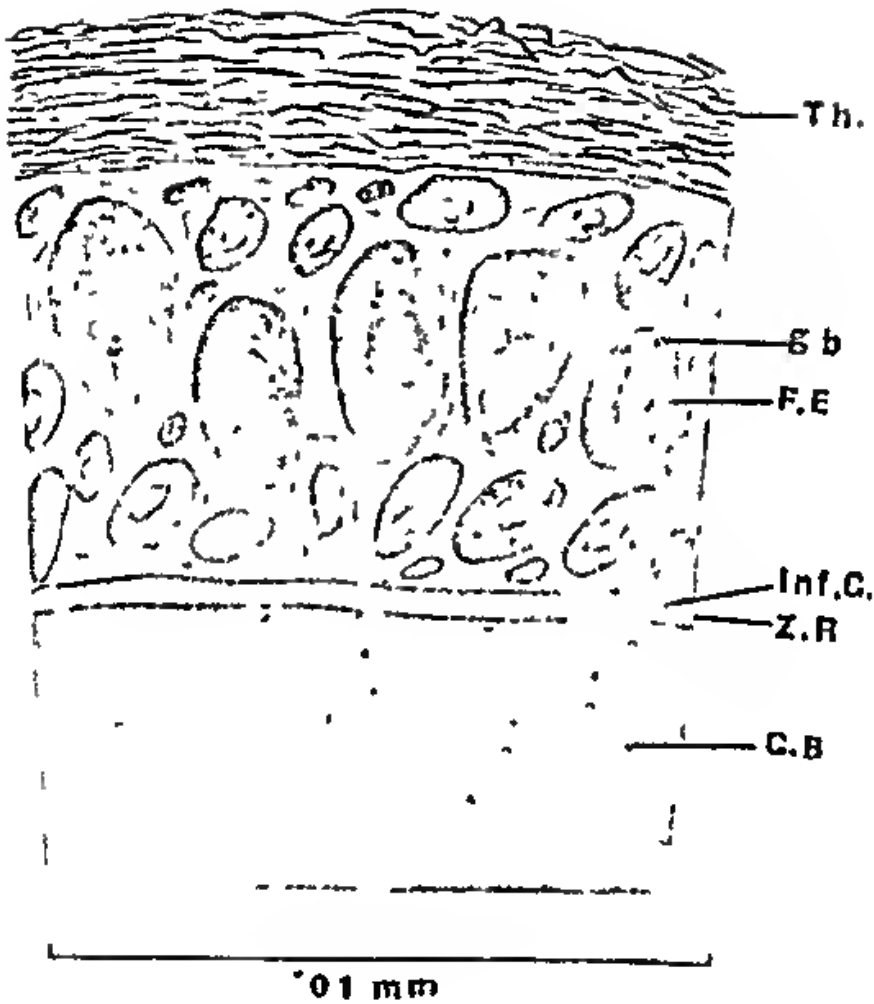

Fig 3

Plate 3

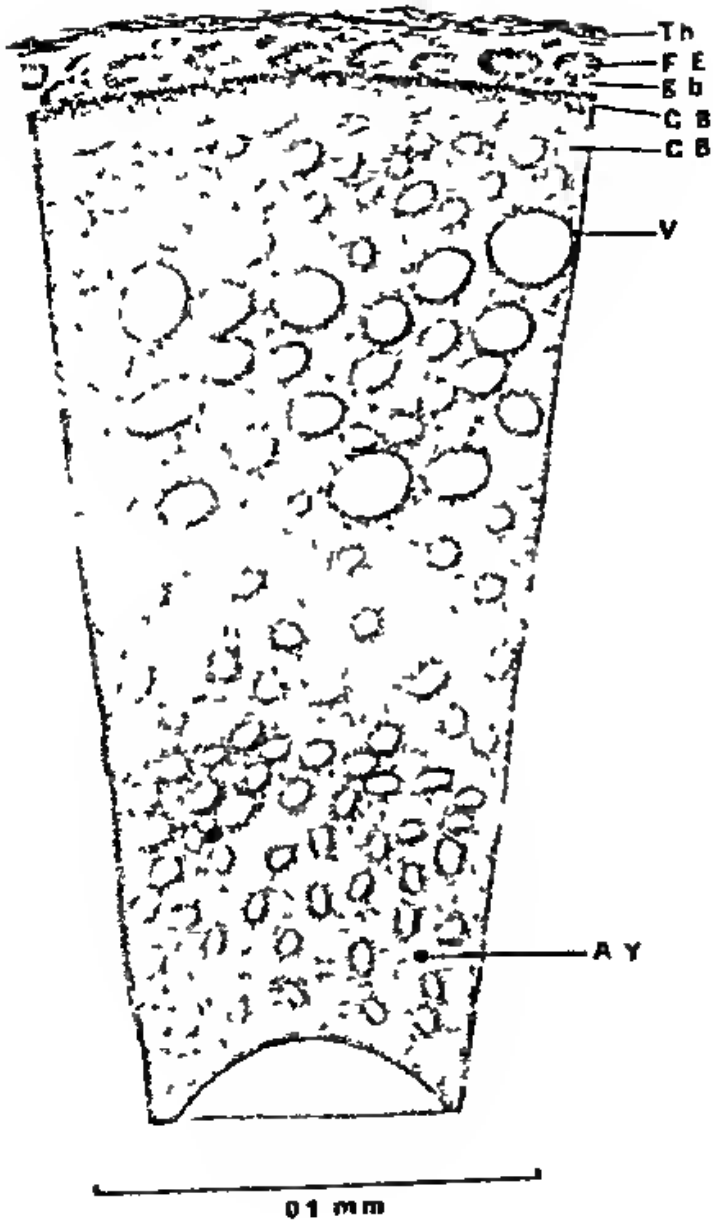

Fig 4

Plate 3

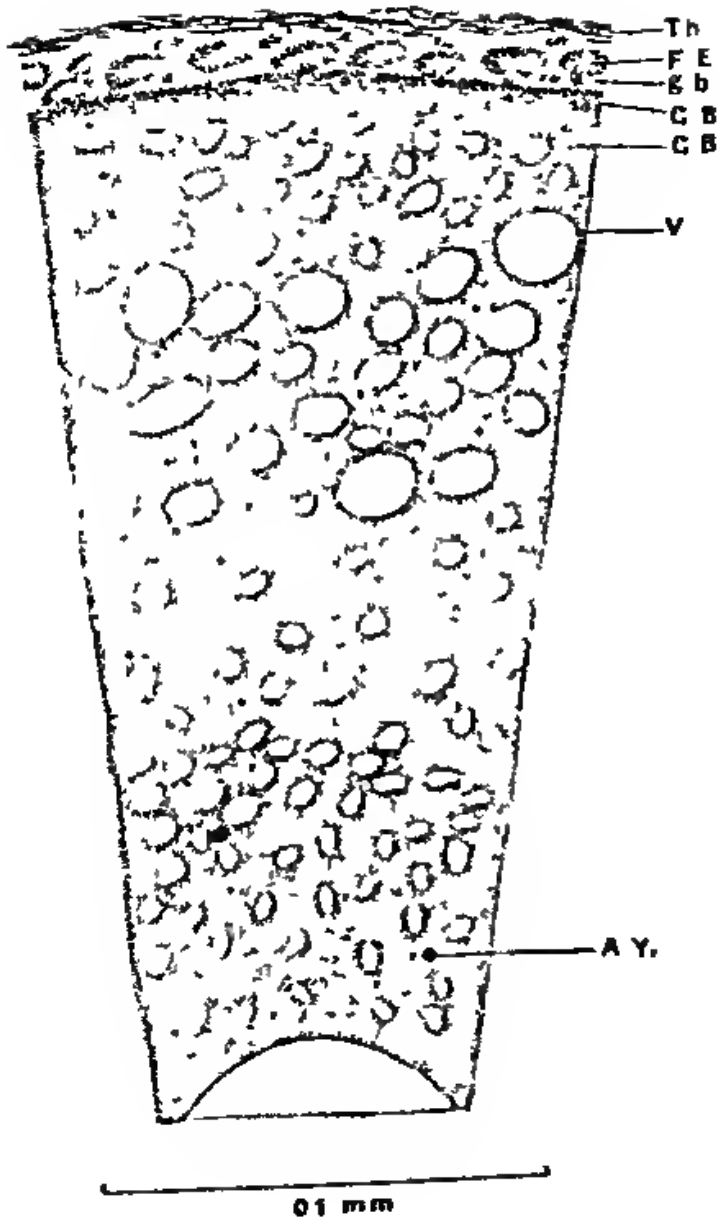

Fig 4

and secondary sexual characters resembling a hen (Fig 1) It was after dissection that we recognised its maleness

Fig 1

Text Fig 1 Photograph of the hen-feathered cock, Ventral aspect It has only one leg—the left, and has plumage and secondary sexual characters resembling a hen $\frac{1}{4}$th of natural size

The body measurements are as follows :—

Length, measured from the tip of the beak to the extremity of the tail .	15 inches
Breadth, round the wings .. .	9 inches
Length of the wing ..	9 5 inches
Length of the normal leg .	13 inches

(measured from the pelvis along the hinder border up to the tip of the third digit)

Length of the femur of the left leg	3 inches
,, ,, Tibio-tarsus .	4 5 inches
,, ,, Tarsometatarsus .	3 inches
,, ,, 1st digit . ..	·75 inch
,, ,, 2nd digit	1 4 inches
,, ,, 3rd digit	2 inches
,, ,, 4th digit .	1 5 inches

For the preparation of the slides of testes, DaFano's cobalt nitrate formol was used for fixation and subsequently the tissue was impregnated with 2 per cent silver nitrate. Sections were cut by paraffin method, toned with gold chloride and stained in iron hæmatoxylin and eosin

THE STRUCTURE OF THE TESTES

The investigations of Benoit, 1921 (1), Firket, 1914 (4), Loisel, 1902 (8), Morgan, 1920 (11), Nonidez, 1920 (12), Pezard, 1918—22 (15), Shattock and Seligmann, 1904 (17), and others have definitely established the fact that the condition of plumage and the development of the secondary sexual characters of a fowl are governed by the internal secretion of its gonads But there still exists diversity of opinion as to the particular cells which produce the secretion in the organ Boring and Morgan (2) have shown that the condition of plumage in cocks of Sebright bantam breed in which all males are hen-feathered, is dependent upon the presence of cells identical with the ovarian type

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of columnar cells towards the periphery of the tubule Scattered among these are rounded cells with oval nuclei—the spermatogonia Transitional stages of the primary and secondary spermatocytes are not uncommon The spermatids and spermatozoa are visible in the course of formation in certain tubules.

Fig. 2

Text Fig 2 Photograph of the bird after the plucking off of feathers lateral view showing complete absence of the right leg (reduced to ⅓th natural size)

The Interstitial Tissue—Ordinarily in the adult cocks the interstitial cells are very scarce In our preparations,

of interstitial cells The secretion from these cells was regarded to inhibit cock-feathering While on the other hand Fell (3) has tentatively put forward a hypothesis that the feathering in the sexes of the fowl might well depend upon the amount of lipoids contained in the blood.

" If the lipoid content is greater than a certain amount, say X, the plumage will be of the female, and if less than X of the male type. In the normal female the amount would be above X, ovariotomy would cause it to fall below X, and the bird would become cock-feathered. In the normal male it would be below X and castration would cause a slight fall, as perhaps expressed by the more luxuriant plumage of the capon In the case of the hen-feathered Sebright and Campine Cocks the fat concentration would be slightly above X, castration would cause it to fall below, and the male plumage would be exhibited."—(Fell, H B., Brit. Journ. Exp Biol, Vol I No 3, 1924, p 307)

In his extensive series of memoirs on the studies of gonads of the fowl, Jose, F. Nonidez (12) has pointed out that in most hen-feathered males the interstitial cells become fat-laden and agrees with the hypothesis advanced by Fell in so far as it assumes that the gonads of either sex stimulate the production of lipoid material in the blood. He remarks—" Although the problem has not been sufficiently studied, the few observations (7 and 13) published thus far are consistent with the hypothesis "

We are unable to determine the lipoid contents in the blood of the bird in question, but on careful histological study of the testes we have been able to ascertain the occurrence of abundant aggregations of large-sized interstitial cells of the ovarian type which according to Morgan and Boring control the expression of secondary sexual characters and also the formation of plumage.

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THE ABNORMALITY OF THE HIND-LEG

We have not been able to find any recorded case of abnormality of the hind-leg of a fowl except the one recently published by one of us (G. N. R) (16). We take the opportunity of describing another in this note

Externally there is no indication of the existence of the right hind-leg (Fig 2). The bird hopped with but one leg. On dissecting the skin the peculiar arrangement of the muscles of the right leg showed clearly the defect to be congenital and not a case of amputation as we failed to discover any scar of healing. All the muscles were degenerate and lumped one above the other as shown in the figure 4 (MP)

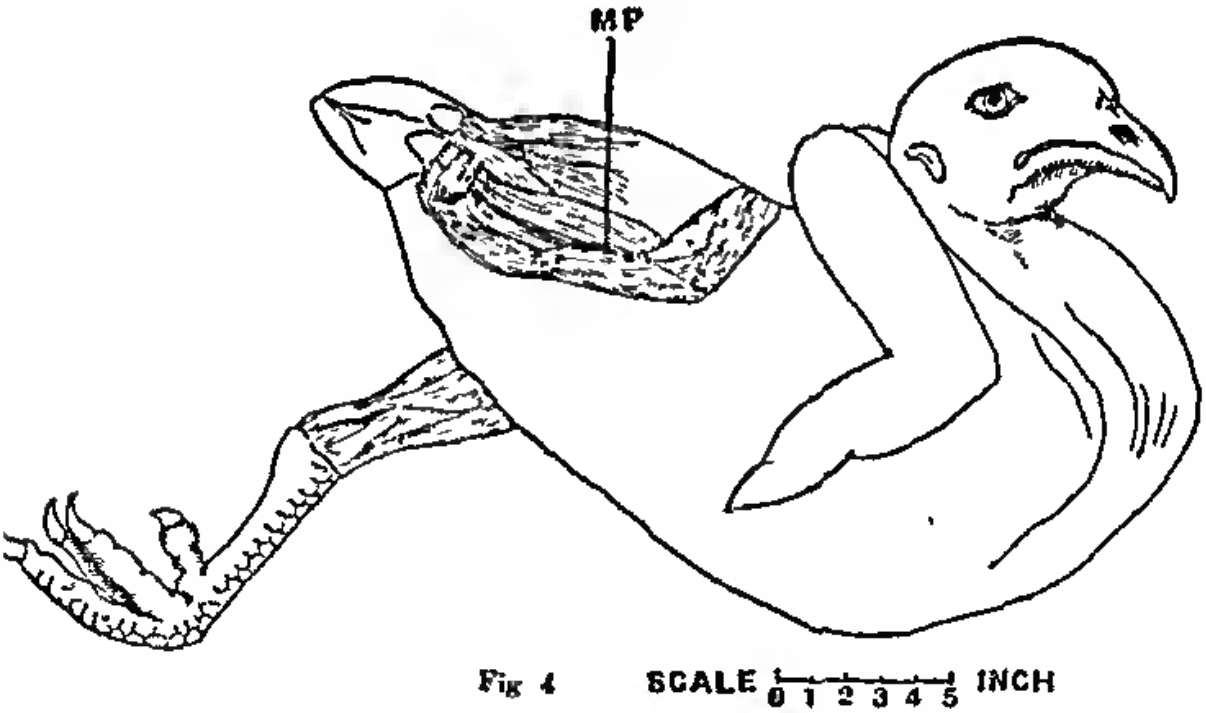

Fig 4

Text Fig 4 Dissection showing the arrangement of muscles of the right side in the pelvic region

MP—Muscles piled up one above the other in the pelvic region of the right side.

however, we were agreeably surprised to find a large number of patches of the interstitial cells They lie chiefly in the large intertubular spaces The cell-outline is very distinct and the nucleus is relatively large, rounded or oval-shaped

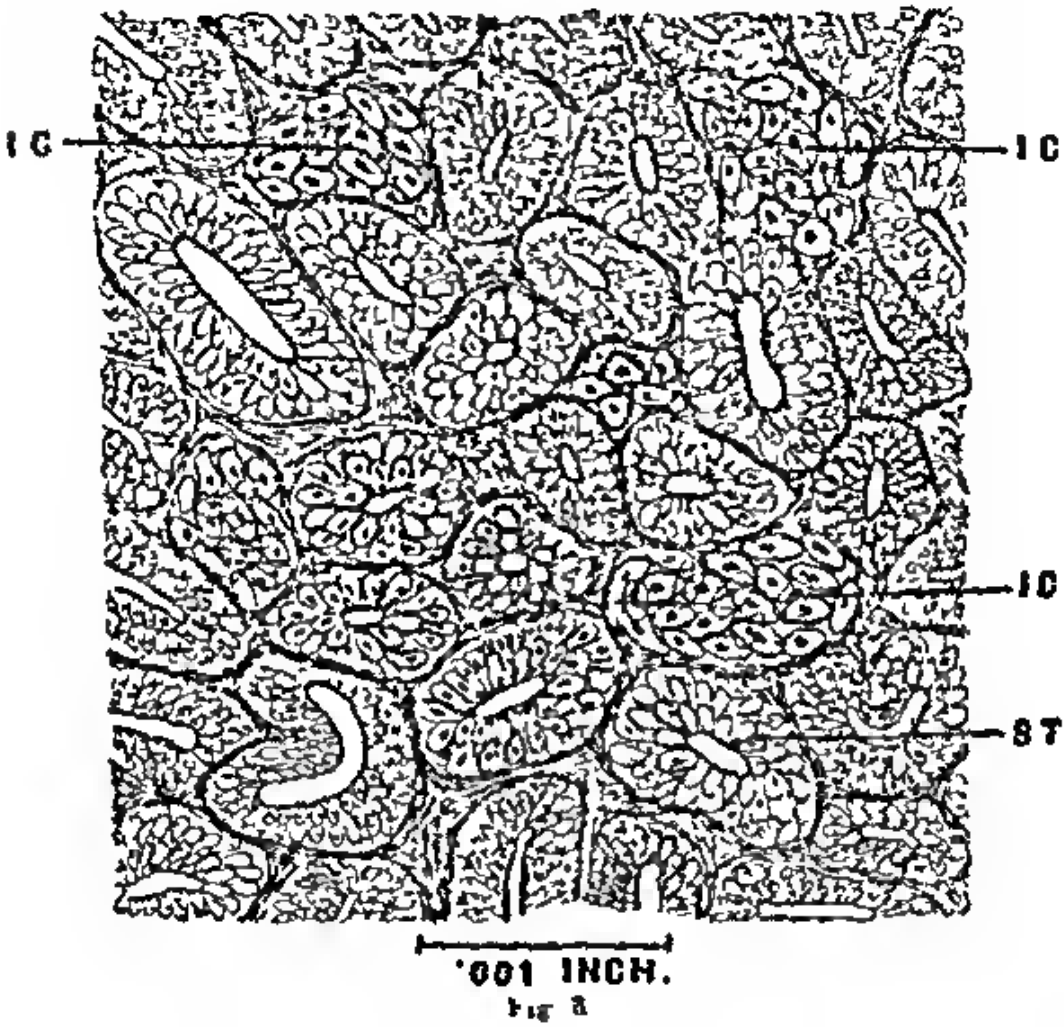

Fig 3

Text Fig 3 Transverse section of the testes showing groups of large interstitial cells in the intertubular spaces

IC—Interstitial cells in the intertubular spaces. ST—Seminal epithelium of the tubule

The cytoplasm is finely granular and contains no vacuole and appears to contain little fat In certain breeds of fowl, however, Prof. Morgan has shown that the interstitial tissue is very abundant in the testes and that the cells become fat-laden These fat-laden cells look to be identical with the so-called luteal cells (Pearl and Boring) or the ovarian type of interstitial cells (Fell, Morgan and

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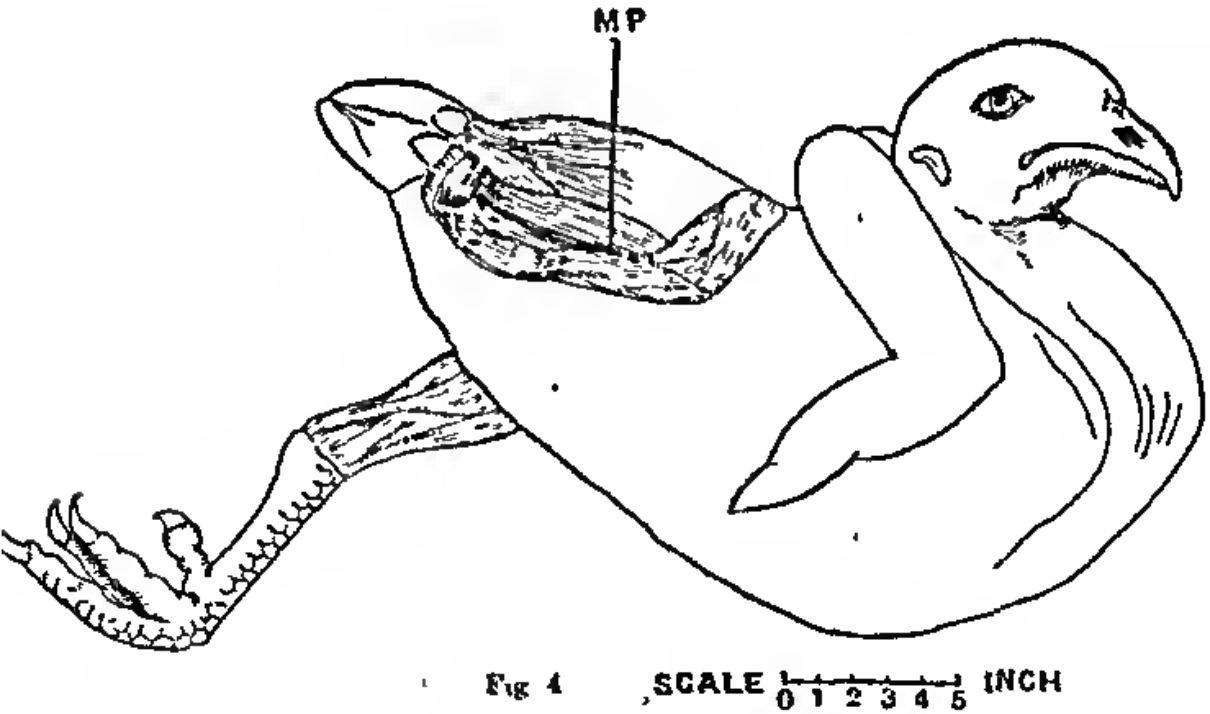

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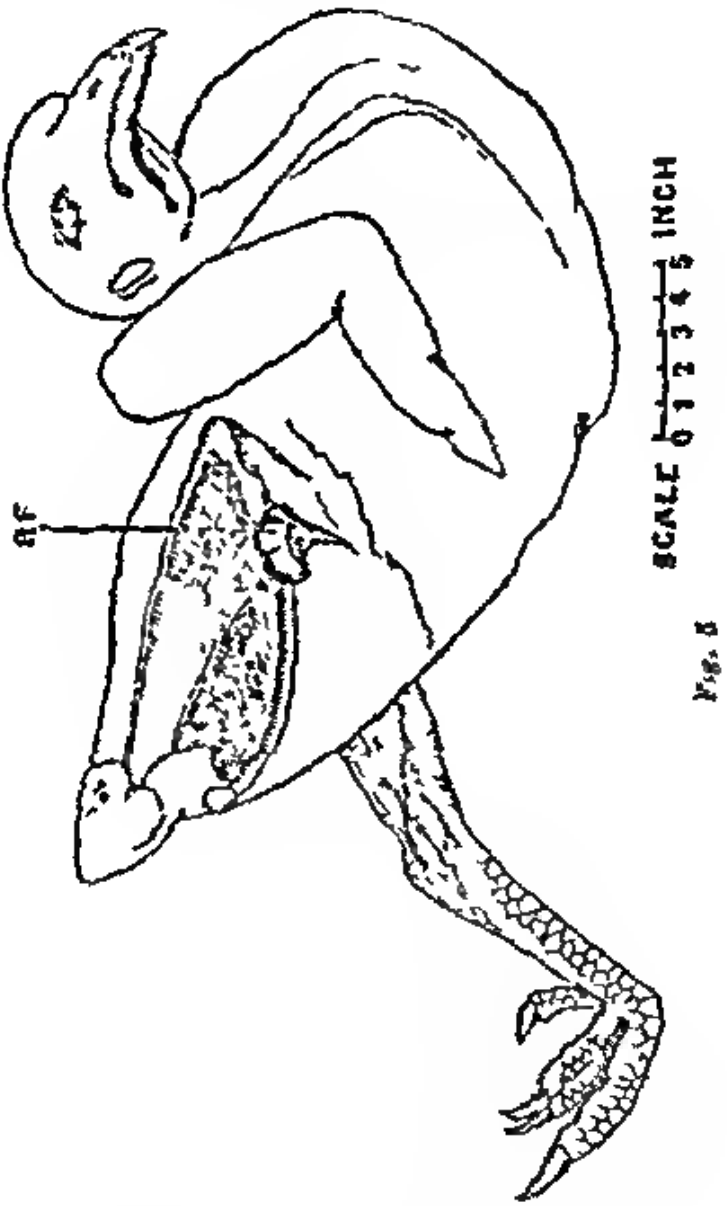
MP—Muscles piled up one above the other in the pelvic region of the right side.

On carefully removing the muscles we came across one tiny nodule of bone about $\frac{1}{8}$th inch in length and $\frac{1}{4}$th inch in breadth attached to the acetabulum Fig 5, RF) This is the whole of the skeleton of the right hind-limb



Text Fig 5 Dissection to show the skeleton of the right hind-leg—a small piece of bone attached to the acetabulum

RF—Skeleton of the right leg, the head is attached to the acetabulum.

The pelvic girdle is expanded and its bony parts are perfectly normal The little nodular bone has got a distinct head like that of the femur which fits in the right acetabulum and forms a ball and socket joint.

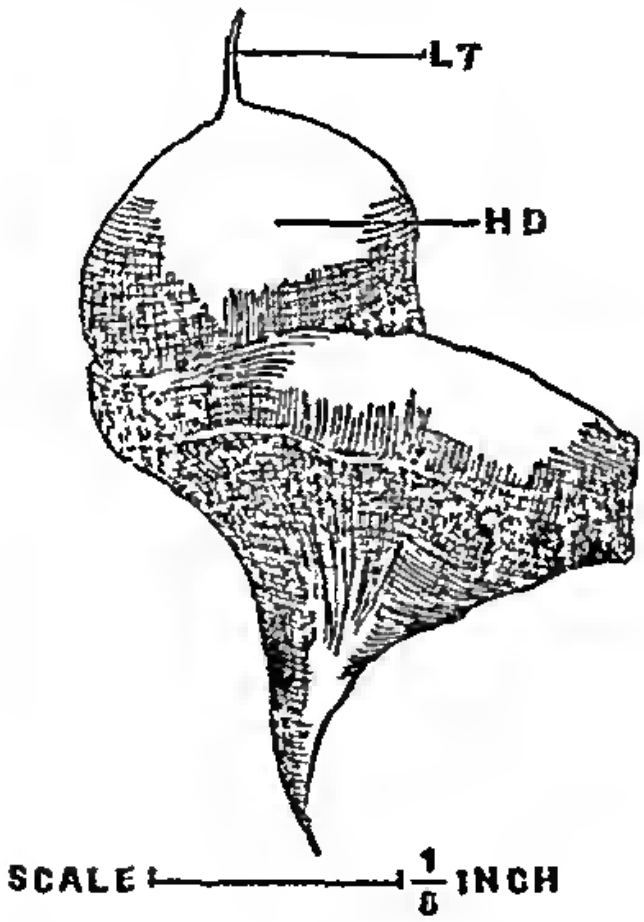


Fig 6

Text Fig 6 The skeleton of the right hind-limb detached from the acetabulum and drawn under a magnifying lens.

LT—Ligamentum Teres. HD—Head.

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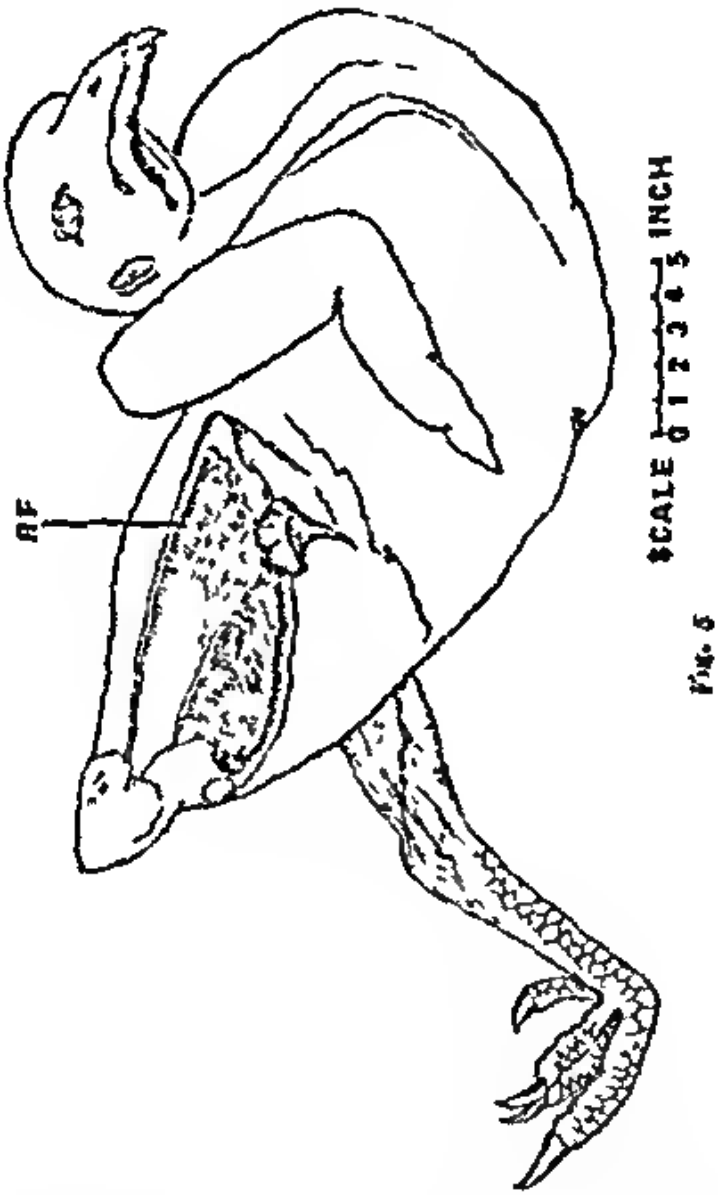

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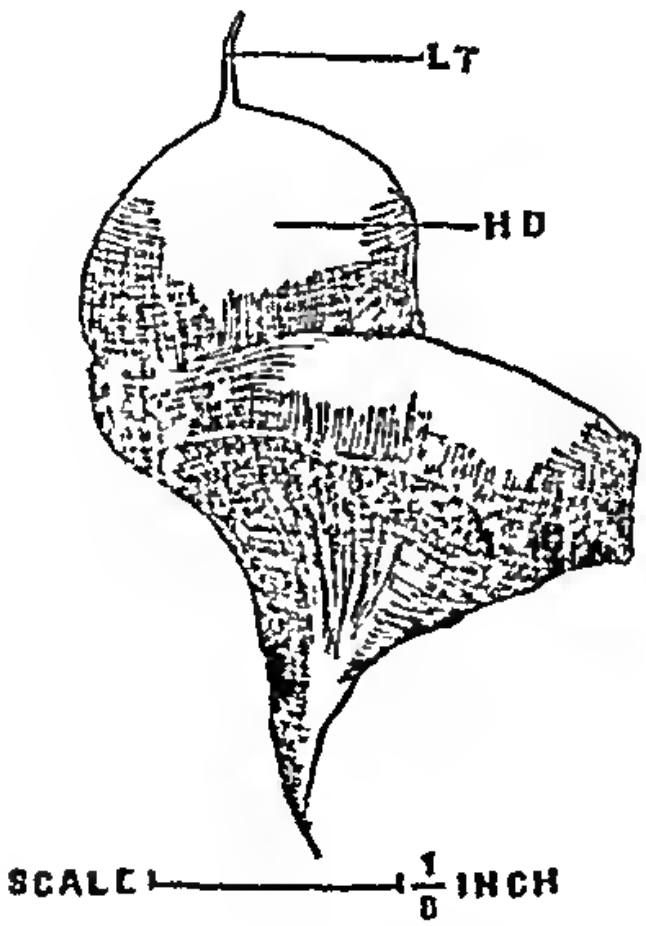

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It has the usual binding ligament—the ligamentum teres (Fig. 6 LT) inserted from the head to the fundus acetabulum. The other ligaments, viz., the capsular, which grasps the brim of the acetabulum and the head of the femur and the Pubo-femoral, binding the pubis and the femur, are wanting.

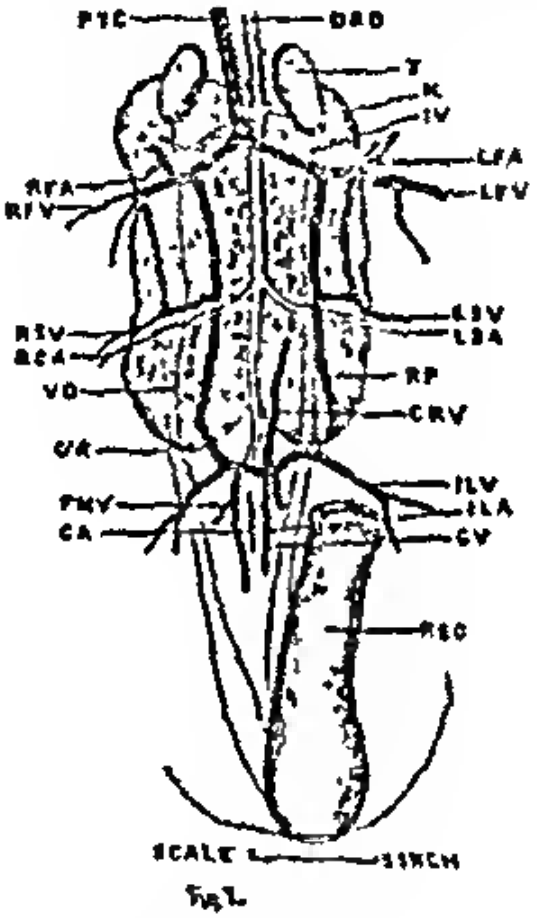

Fig 7

Text Fig 7. Dissection of the arteries and veins of the pelvic region showing the narrow calibre of the right sciatic and femoral arteries and vein

CA—Caudal artery. CMV—Coccygio-mesenteric vein CV—Caudal vein DAO—Dorsal aorta IIA—Internal Iliac artery. IIV—Internal Iliac vein. IV Iliac vein. K—Kidney LFA—Left femoral artery LFV—Left femoral vein. LSA—Left sciatic artery, LSV—Left sciatic vein PMV—Posterior mesenteric vein. PTC—Post Caval vein REC—Rectum RP—Renal Portal vein. RFA—Right femoral artery. RFV—Right femoral vein RSA—Right sciatic artery. RSV—Right sciatic vein. T—Testes. UR—Ureter. VD—Vas deferens

The blood vessels in the pelvic region show normal structure on the left side while on the right they are markedly degenerate. The right femoral artery and vein (Fig 7 RFA, RFV) are extremely narrow in calibre with a very few minute branches The same is the case with the sciatic artery and the vein. These ramifications supply the degenerate muscles of the right leg (MP).

We wish to acknowledge here our thanks to Professor D R Bhattacharya for affording the necessary facilities for work and for offering helpful criticisms.

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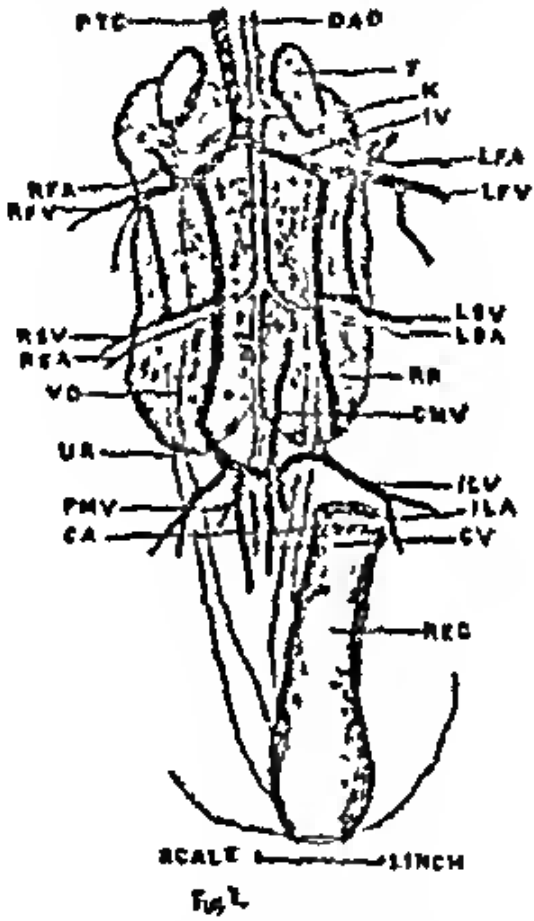

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course both external and internal short branches, which in their turn further branch. The external ramifications extend to the lateral margins of the body. The main excretory canals run into each other by a transverse connection at the anterior end between the oral sucker and the intestinal bifurcation

The testes are much lobed measuring 1 09 mm. in length and 0 49 mm in breadth They are longer than broad and are laterally situated close outside the intestinal cæca with their long axis straight and parallel to the length of the body, unlike the testes of *C. gallinulae* Johnston, which lie obliquely The testes may extend anteriorly a little in the vitelline field. The vasa efferentia arise from about the middle of the inner margin of the testes The vas deferens was not observed on account of the massive vesicula seminalis and the uterine coils The vesicula seminalis is coiled and lies outside the cirrus sac between it and the transverse uterine coils The cirrus sac is median and more or less flask-shaped measuring 0 69 mm in length and 0 21 mm in breadth in the middle of its posterior half. The common genital opening is median and lies immediately behind the intestinal bifurcation

The ovary is lobed and median situated in the intertesticular zone It measures 0 33 to 0·49 mm in length and 0 28 to 0 33 mm in maximum breadth The short wide oviduct arises from the anterior border of the ovary and takes a somewhat S-shaped course before it enters the slightly curved ootype which is surrounded by a fairly large mass of shell gland cells The shell gland mass measures 0 23 mm. in length and 0 28 to 0·34 mm in breadth The Laurer's canal arises from the ootype and opens to the exterior by a small median dorsal pore situated about the level of the posterior margin of the shell gland mass.

The first narrow part of the uterus forms a loop ventrally to the shell gland mass and then continues into a

region, up to the posterior margin of the cirrus sac, it bears minute spiny indentations which hardly project from the surface The ventral papillæ, which are broad at the base and more or less bluntly pointed at the free end, occupy the entire ventral surface of the body and are arranged in regular rows Besides these papillæ there are present the ventral glands arranged in three longitudinal rows between the two intestinal cæca. The glands of the mid-ventral rows lie close to one another forming a continuous line extending from the anterior saccular part of the cirrus sac to about the posterior margin of the ovary The other two rows, which are ventro-lateral in position are composed of a series of seven or eight groups of glands lying more or less separate from one another

The oral sucker is almost terminal, measuring 0 16 to 0 2 mm in diameter A pharynx is absent The œsophagus is short, measuring 0 16 to 0 32 mm in maximum length. The intestinal cæca are almost of the same length, they extend nearly to the hinder end of the body exhibiting small diverticula throughout their length and terminating in a rosette-shaped blind end They lie about half way between the middle line and the body-wall, touching the outer limits of the uterine loops, but near the anterior region of the testes they curve inwards so as to occupy a position between the laterally situated testes and the median ovary

The excretory system is typical of the genus The excretory bladder is almost rounded, and opens to the exterior by a dorsally situated pore near the hinder extremity The cavity of the excretory bladder is funnel-shaped and its inner wall is thrown into six distinct ridges which probably control the excretory opening, forming a structure called "rippon" by Looss. From the excretory bladder two main excretory canals are given off one on each side, which run forwards laterally almost parallel to and close outside the intestinal cæca These canals give off throughout their

Table 1

(A) *Showing the length of the body and of different organs.*

No of specimens	Length of the body	Length of vitellaria	Length of testes	Length of ovary	Length of cirrus sac
1	4·87 mm.	1·95 mm	0·9 mm	0·33 mm.	0·93 mm
2	3·6 mm	1·04 mm	1 mm	0·43 mm	0·69 mm
3	3·34 mm.	1·14 mm.	0·87 mm.	0·42 mm	0·61 mm.
4	3·34 mm	1·04 mm	0·87 mm.	0·45 mm	0·6 mm
5	3·78 mm.	1·45 mm.	0·83 mm	0·49 mm	0·61 mm
6	4·33 mm	1·68 mm	1·08 mm	0·45 mm	0·85 mm.
7	3·94 mm	1·29 mm.	1·00 mm	0·43 mm.	0·65 mm
8	3·76 mm.	1·54 mm	1·08 mm	0·45 mm.	0·69 mm
9	3·6 mm	1·24 mm	1 mm	0·45 mm.	0·64 mm

(B) *Showing the breadth of the body and other organs.*

No of specimens	Breadth of the body in the anterior region of the testes.	Breadth of the testes	Breadth of the ovary	Breadth of the cirrus sac
1.	1·5 mm.	0·44 mm	0·28 mm.	0·21 mm
2	1·21 mm	0·44 mm	0·29 mm	0·16 mm
3	1·32 mm	0·44 mm	0·28 mm	0·15 mm
4	1·17 mm.	0·44 mm	0·28 mm.	0·16 mm
5	1·32 mm.	0·49 mm.	0·33 mm.	0·21 mm.
6.	1·2 mm.	0·49 mm.	0·28 mm.	0·17 mm.
7.	1·27 mm	0·43 mm	0·32 mm.	0·16 mm.
8.	1·29 mm	0·43 mm.	0·29 mm.	0·18 mm.
9	1·2 mm.	0·46 mm.	0·28 mm.	0·15 mm.

series of about twenty transversely placed wide coils which occupy the entire intracæcal region from the anterior end of the testes to the posterior end of the cirrus sac. The uterine coils may overlap to a certain extent the intestinal cæca

The vitellaria, 1·04 to 1·95 mm in length, lie laterally in the middle third of the body, commencing 0·54 mm. distance behind the cirrus sac at about the level of the eighth uterine coil from the anterior end and terminating at the anterior margin of the testes or a little behind it Each vitelline gland consists of twelve groups of two to four follicles each arranged in grape-like bunches. The transverse vitelline ducts arise near the hind end of the vitellaria, they pass between the uterine coils and the ovary and overlap the shell gland mass where they unite in the middle to form a conspicuous *vitelline reservoir* The eggs measure 0·024 to 0·026 mm by 0·011 to 0·013 mm in size and possess at each end a long polar filament which is thicker at the base.

The genus has been recorded for the first time in India. The present species resembles the Australian species *C gallinulae*, Johnston, but differs remarkably from *C. cerrucosa* commonly found in European birds, in the presence of polar filaments of the eggs. It also resembles *C. gallinulæ* in the size of its eggs, but it differs from it in the large size of the vesicula seminalis, large size of the testes, shape of the ovary, and size and shape of the body. The ovary in *C. gallinulæ* is rounded but it is lobed in *C. orientalis* *C. orientalis*, however, resembles *C. cerrucosa* and *C charadrii* in its general shape and size of the body and in the number of uterine loops But it differs from *C charadrii* in the ratio of the length to the maximum breadth of the body which is 3 : 1 as compared to 4 · 1 in *C. charadrii*. It also differs from all the known species mentioned above in the arrangement and number of the ventral glands and papillæ.

situated at about half way between the oral sucker and the middle of the body at about 0·69 to 1·3 mm distance from the anterior end It measures 0·26 to 0·37 mm in diameter

The excretory bladder is Y-shaped The excretory opening is median, situated ventrally, a little in front of the posterior end of the body The genital opening lies a little to the left immediately in front of the ventral sucker and much behind the intestinal bifurcation

The mouth is terminal or slightly sub-terminal A small pre-pharynx is present The pharynx is almost pear-shaped measuring 0·16 mm in length and 0·21 mm in maximum breadth about the middle of its length The œsophagus is a straight tube of moderate length and more or less uniform breadth, measuring 0·1 to 0·21 mm in length Only in one specimen it measured 0·51 mm but the increase in length in this case may be due to much flattening on account of the excessive pressure before fixation The intestinal bifurcation lies almost midway between the oral and ventral suckers at 0·25 mm distance in front of the anterior margin of the acetabulum The intestinal cæca are of unequal length, extending almost up to the posterior extremity, the right cæcum being slightly longer than the left one The cæca are narrower in the anterior half of the body somewhat near the ovary behind which they gradually broaden attaining their maximum breadth of 0·1 mm in the region between the anterior and posterior testes Their course, for the greater part of their length, is almost straight, but they may have a slight bend towards the body-wall in the middle one-third of the body.

The ovary lies in front of the testes, to the right side touching externally or partly covering the right cæcum 0·16 mm distance behind the posterior margin of the ventral sucker. It is almost rounded with entire margin and measures 0·32 to 0·49 mm. in length and 0·37 mm in maximum breadth in its middle region The shell gland complex

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ON A NEW SPECIES OF ASTIOTREMA LSS, ASTIOTREMA-GANGETICUS, WITH A KEY TO THE SPECIES OF THE GENUS

Astiotrema gangeticus sp n

Thirteen specimens of this species were obtained from the duodenum of *Emyda granosa*, the tortoises dissected at Allahabad Only one out of four was found to be infected with these parasites and a species of the genus *Cephalogonimus*

The distomes were attached to the wall of the duodenum in the anterior one-third of its entire length. The parasites when kept alive in 0 75 per cent salt solution lived only for about forty-eight to fifty hours

The body is almost oval or somewhat elliptical, measuring 4 to 6 7 mm in length when flattened under the pressure of a cover-glass The breadth varies in different regions as will be seen from the Table 2, but the maximum breadth lies at the anterior region of the anterior testis measuring 2 1 mm Both anterior and posterior extremities are almost rounded, specially the latter, which is always broader Spines are present only on the ventral surface, where they are arranged in regular transverse rows The presence of spines only on the ventral surface is a characteristic feature of this species The suckers are spherical and the ratio in their size is 4 : 5. The oral sucker lies at the anterior end or a little behind it, facing ventrally, and measures 0 18 to 0·28 mm in diameter The ventral sucker is median and

large convoluted knot in the posterior third of the body. The metraterm runs about the median plane of the body inwards to the cirrus sac, passing dorsally to the acetabulum to open into the genital atrium The eggs are oval in shape, measuring 0·042 mm in length and 0·017 mm in breadth

The vitellaria lie outside the intestinal cæca, nearer them than the body-wall. The vitellaria commence behind the ventral sucker from about the beginning of the second quarter and terminate at about the end of the third quarter of the body. Each vitelline gland consists of a large number of follicles which lie close to one another in a continuous series, not in definite groups as in other species.

The species is characterised by the elliptical shape of the body, presence of spines on the ventral surface only, large size of the receptaculum seminalis, the ventral sucker being larger than the oral sucker, greater length of the intestinal cæca in proportion to the length of the body, the vitellarian follicles forming a more or less continuous chain and not divided up into separate grape-like bunches, large size of the cirrus sac, rounded form of the ovary, and the ventral subterminal position of the excretory opening It, however, resembles *Ast loossii* in the position and form of the testes, shape of the cirrus sac, the length of the œsophagus and intestinal cæca, elliptical form of the body and the ventral position of the excretory opening

KEY TO THE SPECIES OF THE GENUS ASTIOTREMA LSS.

The key to the species of the genus *Astiotrema* Lss as given by Mehra is modified here in order to include the present species and the emended key is as follows :

Ovary lobed ... ... *Ast. loossii.*

occupies nearly a median position at about the level of the inner margin of the posterior half of the ovary. The receptaculum seminalis is large and has more or less an elongated saccular form, measuring 0 92 mm in length and 0 29 mm in maximum breadth in its posterior half In most cases it occupies an oblique position just behind the ovary covering dorsally the right intestinal cæcum and approaching the vitellarian follicles of that side The Laurer's canal is a small narrow tube which runs parallel to the anterior half of the receptaculum seminalis and then bends outwards to open to the exterior slightly to the left of the mid-dorsal line a little behind the level of the shell gland mass

The testes are much lobed They are situated in the middle one-third of the body-length. In some specimens, however, the posterior testis may extend a little into the posterior third of the body The anterior testis lies a little distance behind the ovary to the left side touching the inner wall of the left intestinal cæcum It measures 0 58 to 0 93 mm in length and 0·52 to 0 8 mm in maximum breadth. The posterior testis is slightly larger showing the same range of variation in size as the anterior testis The vasa efferentia arise from the middle of the anterior margin of the testes and unite to form the vas deferens at the level of the middle of the ovary The vas deferens is of moderate length and runs parallel to the metraterm The cirrus sac has thick muscular walls, measuring 1 06 mm. in length and 0 33 mm in maximum breadth about the middle of its saccular part. It extends far behind the acetabulum, as far back as the middle of the ovary, with its long axis median, to the right or to the left side of the median line and parallel to the length of the body. Its narrow tubular terminal part lies dorsally to the right or left side of the ventral sucker except near the genital opening

The uterus is much convoluted and both its ascending and descending parts pass between the testes forming a

large convoluted knot in the posterior third of the body. The metraterm runs about the median plane of the body inwards to the cirrus sac, passing dorsally to the acetabulum to open into the genital atrium. The eggs are oval in shape, measuring 0 042 mm in length and 0 017 mm. in breadth

The vitellaria lie outside the intestinal cæca, nearer them than the body-wall. The vitellaria commence behind the ventral sucker from about the beginning of the second quarter and terminate at about the end of the third quarter of the body Each vitelline gland consists of a large number of follicles which lie close to one another in a continuous series, not in definite groups as in other species.

The species is characterised by the elliptical shape of the body, presence of spines on the ventral surface only, large size of the receptaculum seminalis, the ventral sucker being larger than the oral sucker, greater length of the intestinal cæca in proportion to the length of the body, the vitellarian follicles forming a more or less continuous chain and not divided up into separate grape-like bunches, large size of the cirrus sac, rounded form of the ovary, and the ventral subterminal position of the excretory opening It, however, resembles *Ast. loossi* in the position and form of the testes, shape of the cirrus sac, the length of the œsophagus and intestinal cæca, elliptical form of the body and the ventral position of the excretory opening

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The key to the species of the genus *Astiotrema* Lss. as given by Mehra is modified here in order to include the present species and the emended key is as follows :

Ovary lobed ... . *Ast. loossi.*

occupies nearly a median position at about the level of the inner margin of the posterior half of the ovary. The receptaculum seminalis is large and has more or less an elongated saccular form, measuring 0·92 mm in length and 0·29 mm. in maximum breadth in its posterior half. In most cases it occupies an oblique position just behind the ovary covering dorsally the right intestinal cæcum and approaching the vitellarian follicles of that side. The Laurer's canal is a small narrow tube which runs parallel to the anterior half of the receptaculum seminalis and then bends outwards to open to the exterior slightly to the left of the mid-dorsal line a little behind the level of the shell gland mass.

The testes are much lobed. They are situated in the middle one-third of the body-length. In some specimens, however, the posterior testis may extend a little into the posterior third of the body. The anterior testis lies a little distance behind the ovary to the left side touching the inner wall of the left intestinal cæcum. It measures 0·58 to 0·93 mm. in length and 0·52 to 0·8 mm. in maximum breadth. The posterior testis is slightly larger showing the same range of variation in size as the anterior testis. The vasa efferentia arise from the middle of the anterior margin of the testes and unite to form the vas deferens at the level of the middle of the ovary. The vas deferens is of moderate length and runs parallel to the metraterm. The cirrus sac has thick muscular walls, measuring 1·06 mm. in length and 0·33 mm. in maximum breadth about the middle of its saccular part. It extends far behind the acetabulum, as far back as the middle of the ovary, with its long axis median, to the right or to the left side of the median line and parallel to the length of the body. Its narrow tubular terminal part lies dorsally to the right or left side of the ventral sucker except near the genital opening.

The uterus is much convoluted and both its ascending and descending parts pass between the testes forming a

(B)

No of specimens	At the end of vitellaria	At the ant end near the ant. margin of the oral sucker	At the post. end near the exc pore.
1.	1 67 mm	0 42 mm.	0 42 mm
2	1 85 mm	0 42 mm	0·58 mm
3.	0 98 mm	0 53 mm	0·64 mm
4.	1·59 mm	0 68 mm	0 68 mm
5.	1 82 mm	0 67 mm.	1·06 mm.
6.	1·82 mm	*0 19 mm*	0 74 mm
7.	2 00 mm.	0 2 mm.	0 74 mm

Table 3

Showing length of the body, the length and breadth of reproductive and digestive organs.

No of specimens.	Length of ovary.	Length of vitellaria.	Length of œsophagus	Length of pharynx	Length of cirrus sac.	Length of ant. testis.
1	4 5 mm	2 54 mm	0 1 mm	0 05 mm	0·70 mm	0 67 mm.
2	4·7 mm	2 44 mm	0 2 mm	0 1 mm	0·88 mm	0 64 mm
3	4·0 mm	2 35 mm	0 1 mm	0 1 mm	1 07 mm	0·66 mm
4	4 31 mm	2 54 mm	.	0·1 mm	0 70 mm	0 58 mm
5	5·24 mm.	2·70 mm	.	..	...	0 69 mm.
6	6 19 mm.	3 65 mm.	..	...	. .	0 93 mm
7.	6·72 mm	3 61 mm.		. .	.	0 92 mm

Ovary entire—

(1) Intestinal bifurcation at posterior margin of ventral sucker . *Ast. monticelli.*

(2) Intestinal bifurcation in front of the ventral sucker—

(*a*) Vitellaria terminating at middle of the anterior testis ... *Ast implectum*

(*b*) Vitellaria terminating behind the anterior testis—

(i) Oral sucker slightly smaller than the ventral sucker .. *Ast gangeticus Sp n*

(ii) Oral sucker slightly larger than the ventral sucker—
Diameter of suckers—0 25-0 3 mm , testes broader than long—*Ast. reniferum* ;
Diameter of suckers—0 36-0 62 mm. ; testes longer than broad —*Ast elongatum*

TABLE 2

Showing the breadth of the body in different regions.

(A)

No of specimens	At the middle region	At the ant margin of ant testis.	At the ant. margin of acetabulum	At the post margin of the post testis
1	2 mm.	1·93 mm	1·64 mm	1·85 mm
2.	1·9 mm.	1·8 mm.	1·49 mm	1·87 mm.
3.	1·9 mm.	1·8 mm	1·43 mm	1·38 mm
4	1·74 mm	1·73 mm.	1·54 mm.	1·61 mm
5	2·1 mm.	2 mm	1·43 mm	1·97 mm
6	2·08 mm.	2·08 mm.	1·69 mm.	1·96 mm
7	2 mm	2 mm.	1·63 mm.	1·92 mm

(B)

No of specimens	At the end of vitellaria	At the ant. end near the ant margin of the oral sucker.	At the post. end near the exc pore
1.	1 67 mm.	0 42 mm.	0 42 mm.
2.	1 85 mm.	0 42 mm	0·58 mm
3.	0 98 mm.	0 53 mm	0·64 mm
4.	1·59 mm.	0 66 mm.	0·68 mm
5.	1 82 mm.	0 67 mm.	1 06 mm
6.	1·82 mm.	*0 19 mm*	0 74 mm
7.	2·00 mm.	0 2 mm.	0·74 mm

Table 3

Showing length of the body, the length and breadth of reproductive and digestive organs.

No of specimens.	Length of ovary.	Length of vitellaria.	Length of œsophagus	Length of pharynx	Length of cirrus sac.	Length of ant. testis.
1.	45 mm	2·54 mm	0·1 mm	0 05 mm	0·70 mm.	0·67 mm
2.	47 mm	2 44 mm	0·2 mm.	0·1 mm	0·88 mm.	0 64 mm
3.	40 mm	2 35 mm.	0·1 mm.	0·1 mm	1·07 mm	0·66 mm.
4.	4·31 mm.	2·54 mm.	..	0·1 mm	0·79 mm	0 58 mm.
5	5·24 mm.	2·70 mm	.	...	...	0·69 mm
6.	6·19 mm.	3·65 mm.	...	...	...	0·93 mm.
7.	6·72 mm.	3·81 mm	..	..	.	0·92 mm

Ovary entire—

(1) Intestinal bifurcation at posterior margin of ventral sucker . *Ast. monticelli.*

(2) Intestinal bifurcation in front of the ventral sucker—

(*a*) Vitellaria terminating at middle of the anterior testis ... *Ast. implectum.*

(*b*) Vitellaria terminating behind the anterior testis—

(i) Oral sucker slightly smaller than the ventral sucker .. *Ast gangeticus Sp n.*

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Diameter of suckers—0 25-0 3 mm ; testes broader than long—*Ast. reniferum* ;

Diameter of suckers—0 36-0 62 mm. ; testes longer than broad —*Ast elongatum*

TABLE 2

Showing the breadth of the body in different regions.

(A)

No of specimens	At the middle region	At the ant margin of ant testis	At the ant margin of acetabulum	At the post margin of the post testis
1	2 mm	1·98 mm.	1 64 mm	1·85 mm
2.	1 9 mm.	1·8 mm	1 49 mm	1 87 mm.
3	1 9 mm	1·8 mm	1 43 mm	1 38 mm
4	1·74 mm	1 73 mm.	1 54 mm	1 61 mm
5	2 1 mm	2 mm.	1 43 mm	1·97 mm
6	2 08 mm.	2·03 mm.	1·69 mm	1·96 mm
7	2 mm	2 mm	1 63 mm.	1·92 mm

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EXPLANATION OF FIGURES

Fig 1—Ventral View of *Astiotrema gangeticus*

Fig 2—Transverse Section of *Ast gangeticus* passing through the Region of the Ovary.

Fig. 3—Longitudinal horizontal Section of *Ast gangeticus*

Fig 4—Digrammatic Sketch of the Female Reproductive Organs as Constructed from the Series of Transverse Sections

Fig 5—Sketches Showing the Expansion of the Excretory Bladder in Living *Catatropis orientalis*

Fig 6—Ventral View of *Catatropis orientalis* Showing the General Anatomy

Fig 7—Digrammatic View of *C orientalis* Showing the Arrangement of the Ventral Glands

Fig 8—Transverse Section of *C orientalis* passing through the Region of Testes

Fig 9—Digrammatic Sketch of the Female Reproductive Organs as Constructed from the Series of Transverse Sections

No. of specimens	Length of post testis	Length of ovary	Breadth of the cirrus sac in the middle region	Breadth of ant testis	Breadth of post. testis	Breadth of ovary.
1.	0·64 mm	0·37 mm	0·23 mm	0·58 mm.	0·64 mm	0·29 mm
2	0·65 mm	0·37 mm	0·32 mm	0·53 mm	0·72 mm	0·29 mm
3	0·63 mm	0·32 mm	0·32 mm	0·68 mm.	0·71 mm	0·27 mm
4	0·90 mm	0·42 mm.		0·85 mm	0·74 mm.	0·35 mm
5	0·63 mm	0·32 mm	0·32 mm	0·68 mm	0·71 mm	0·35 mm.
6	1·02 mm	0·49 mm		0·82 mm.	0·85 mm.	0·35 mm
7	0·95 mm	0·47 mm		0·80 mm	0·77 mm	0·37 mm.

Plate 1

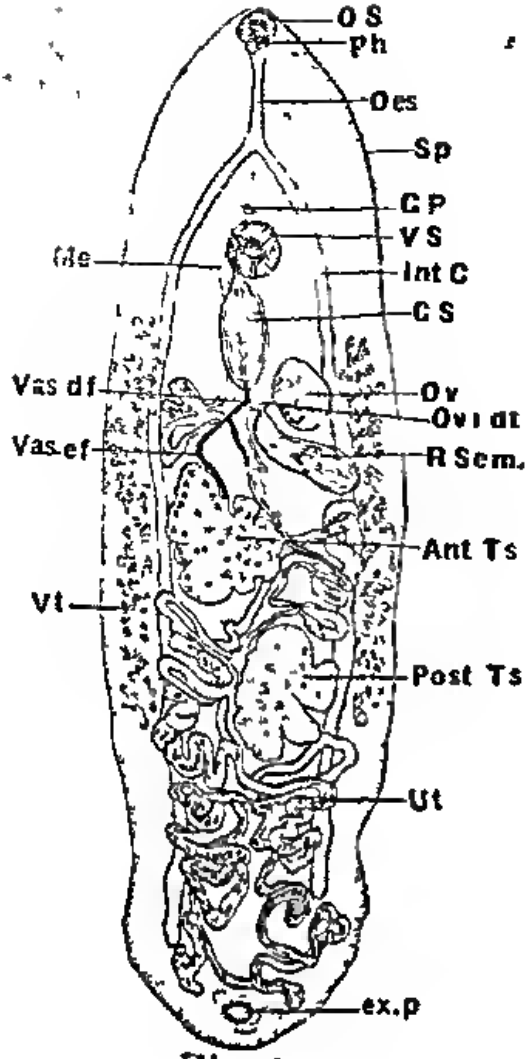

Fig 1.

Dorsal View

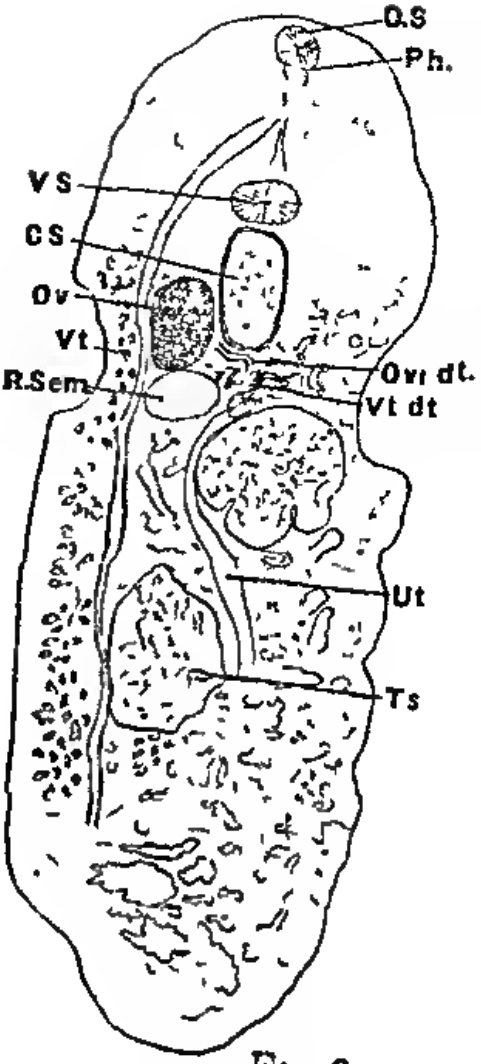

Fig 3

1 mm

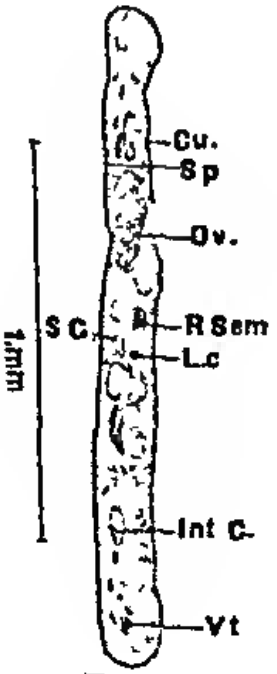

Fig 2.

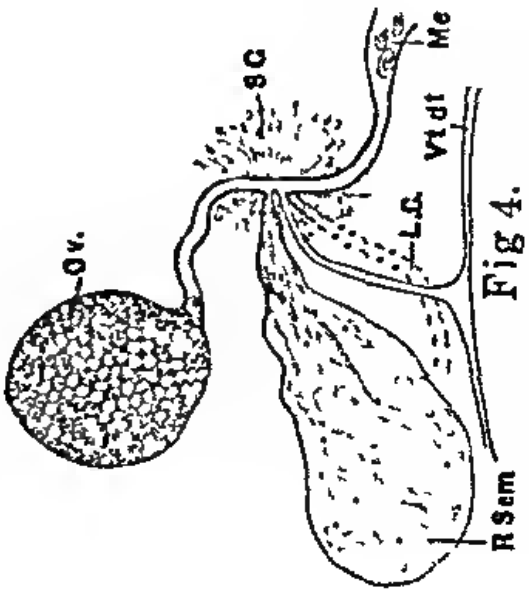

Fig 4.

EXPLANATION OF LETTERING

Ant Ts	..	anterior testis
c. s		Cirrus sac
ex B.		Excretory bladder
ex. c.	.	excretory canal
ex p	.	excretory pore
G P.	.	*genital pore*
Int c		intestinal cæcum
L. c		Laurer's canal
Me	...	metraterm
Oes	.	oesophagus
O S	.	oral sucker
Ov	..	ovary
Ovi Dt	.	oviduct
Ph.	..	pharynx
Post Ts		posterior testis
R S	.	receptaculum seminalis
S G	..	shell gland
Sp	.	ventral space
Ts		testis
Ut		uterus
Vas df.		vas deferens
Vas ef.	.	vas efferens
V S		ventral sucker.
Vt		vitellaria
Vt dt.	..	vitelline duct
Vt. R	.	vitelline reservoir.
V Sem.	..	vesicula seminalis
Cu	.	cuticle
V G	..	Ventral gland

Plate 1

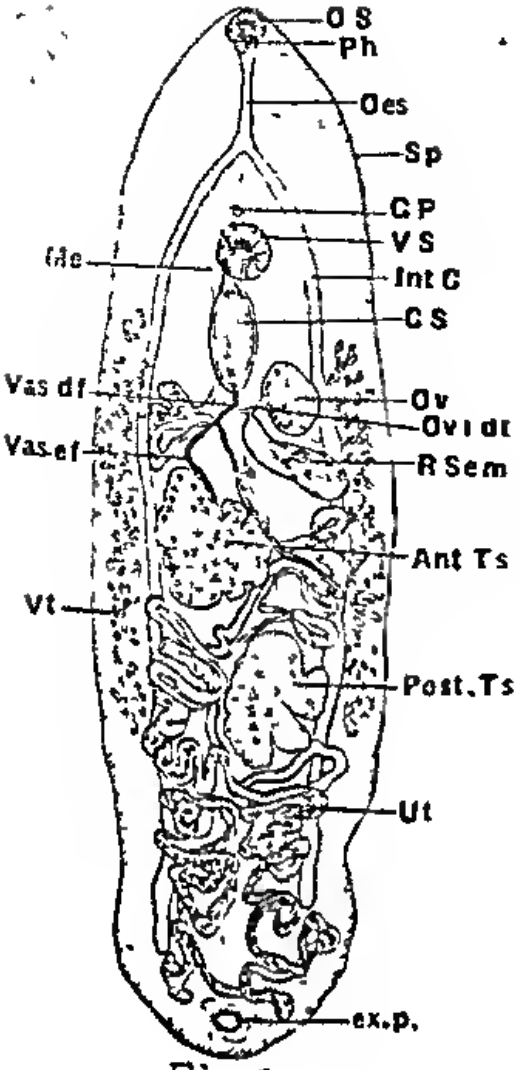

Fig 1.

Dorsal View

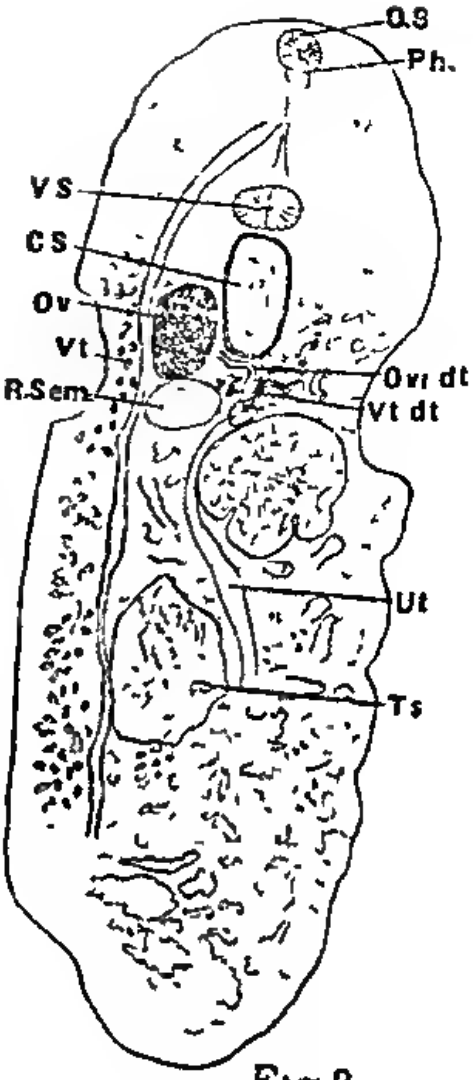

Fig 3

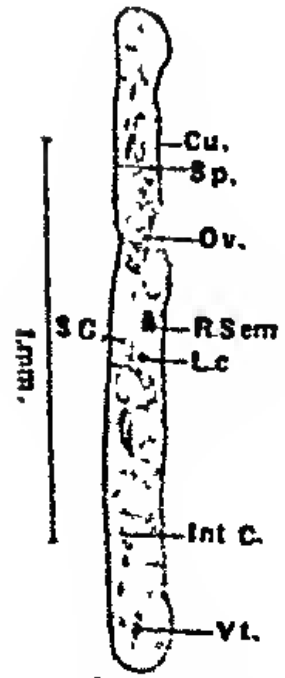

Fig 2

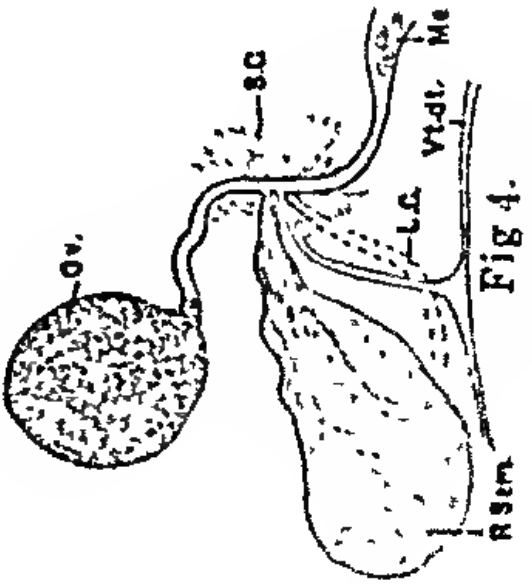

Fig 4.

Plate 2

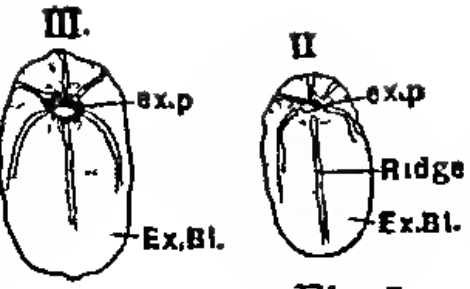

Fig 5

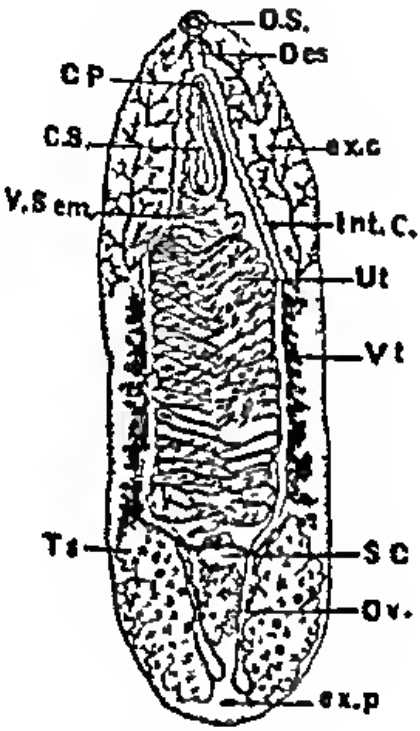

Fig.6.

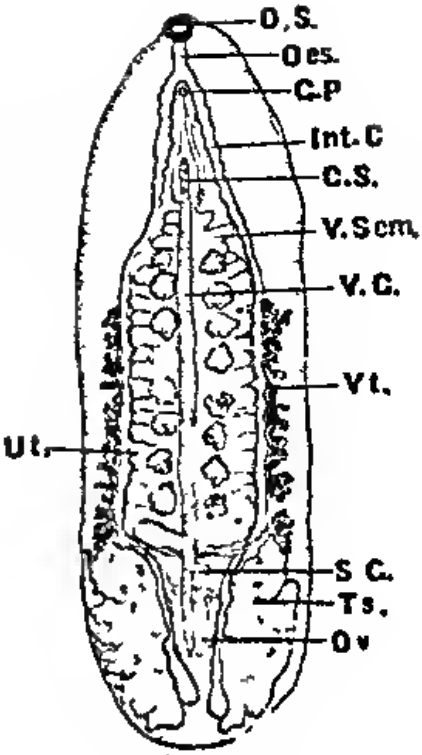

Fig.7.

Fig.9

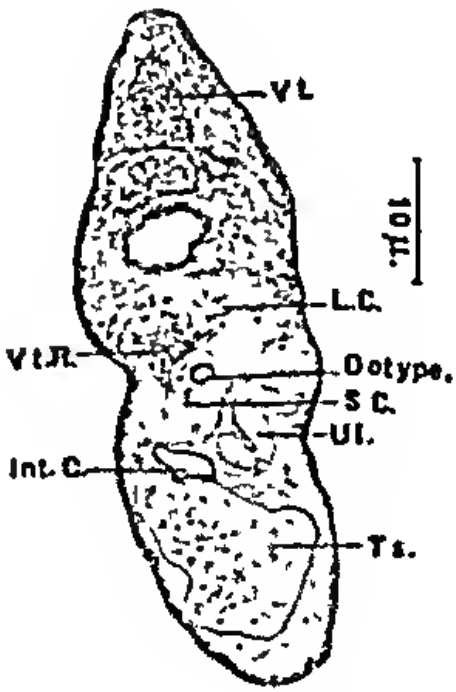

Fig.8.

Plate 2

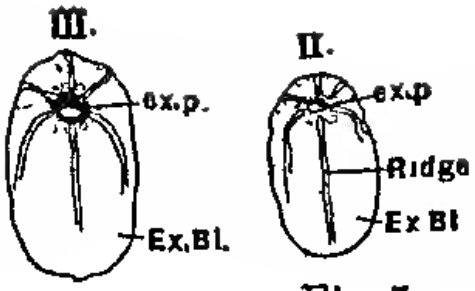

Fig 5

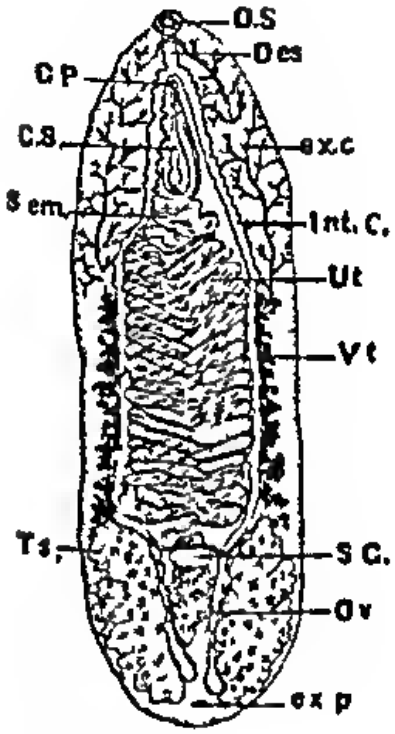

Fig.6.

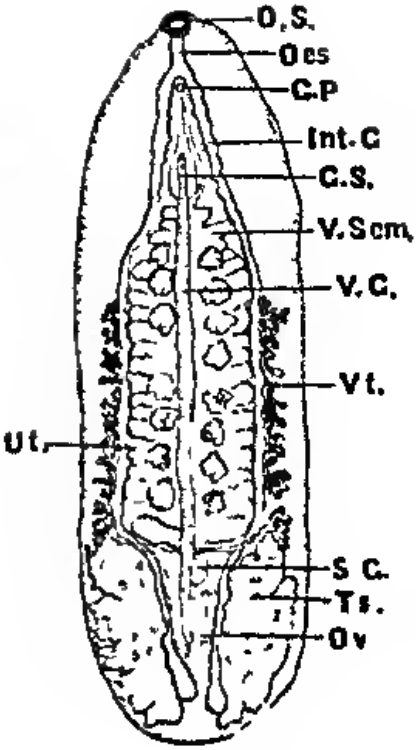

Fig.7

Fig 9

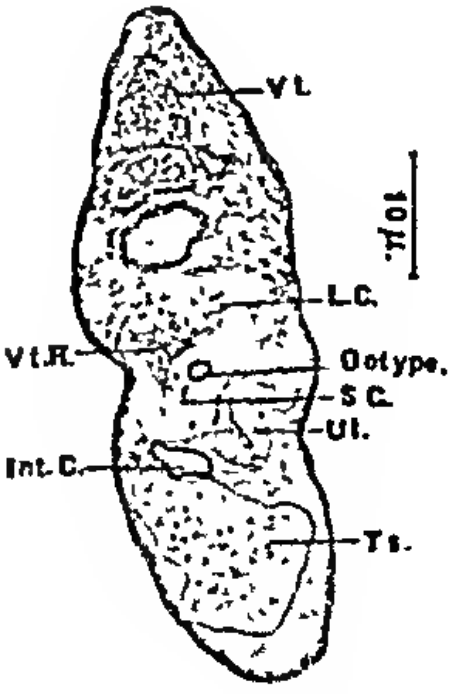

Fig 8.

ON NEW DISTOMATE TREMATODES OF THE SUB-FAMILY TELORCHIINAE (FAMILY LEPODERMATIDAE) WITH A SYSTEMATIC DISCUSSION OF ITS GENERA

BY

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The distomes of the sub-family Telorchiinæ have received a considerable attention by the various workers on the group in Europe, America, and Australia but we have had no account of any Indian species till now The present paper deals with a new genus *Paracercorchis* commonly found in a fresh water tortoise *Kachuga dhongoka* at Allahabad and a new species of the genus *Cercorchis* also met with in the same host.

Perkins in 1928 created two new genera, *Lecithopyge* and *Cercolecithos* for *Opisthioglyphe rastellum* Olsson and *Cercorchis arrectus* Molin respectively including them along with *Brachysaccus* and *Dolichosaccus* in the sub-family *Telorchiinæ. Travassos in 1930 has combined Opisthioglyphe* and *Brachysaccus* in the genus *Opisthioglyphe*, and *Lecithopyge* and *Dolichosaccus* in the genus *Dolichosaccus*, thus reducing the number of these genera from four to two only. While we agree with Travassos in assigning *Lecithopyge rastellum* to *Dolichosaccus*, we maintain that *Opisthioglyphe* and *Brachysaccus* should be recognised as separate genera. The genus *Opisthioglyphe*, which has

been included by some authors in the sub-family Lepodermatinæ must be assigned to the sub-family Telorchiinæ as it closely resembles the genera *Brachysaccus* and *Dolichosaccus*—a fact which was pointed out before by Johnston (1912), Perkins (1928), and Travassos (1930)

Stunkard in 1916 combined the genera *Telorchis* and *Cercorchis* in one genus called *Telorchis*, but Perkins in 1928 following the previous workers, *i e*, Looss and Lühe separated and recognised them again as separate genera which he defined While we are in agreement with the latter author in this respect, we find that *Paracercorchis*[1] nov gen combines in itself several important features of the genera *Cercorchis* and *Telorchis*, and also throws some light on the relationships of the genera *Prolenes* and *Cercolecithos*

The sub-family Telorchiinæ belongs no doubt to the family Lepodermatidæ as discussed by one of us in 1931 The Y-shaped excretory bladder with a long median stem, the cirrus sac with its contained organs, and the position of the ovary and that of the genital pore in the Telorchiinæ are very similar to those of the typical Lepodermatidæ, but the position of the testes behind the uterus and near the hinder end of the body sharply separates this sub-family from the other sub-families of the Lepodermatidæ Though in *Telorchis* and some species of *Opisthioglyphe* and *Dolichosaccus*, the testes lie more forward midway between the genital aperture and the posterior end of the body, the uterus does not extend behind the testes to the hinder end as in the other sub-families of the Lepodermatidæ

The cirrus sac and metraterm are exceedingly long and coiled in *Cercorchis dhongokii*—a feature which gives this species a unique distinction in the Telorchiinæ, but as it resembles in almost all other points the other species of the genus *Cercorchis*, the great length of the cirrus sac

[1] We assign *Telorchis parma* (Braun) to *Paratelorchis* nov gen

and metraterm cannot be considered as characters of more than specific rank.

Paracercorchis Pellucidus nov. gen , nov. sp

Out of thirty specimens of *Kachuga dhongoka* examined during 1930, only two were found to contain within the upper part of their small intestine forty mature specimens of this parasite One immature specimen was also obtained in 1929 from the same host

The distome is blackish in colour in the middle third of the body on account of the vitellaria and the innumerable eggs contained within the uterus, but the anterior and the posterior regions are greyish white In entire mounts the specimens measure 7·12 mm in length and 1·7 mm. in breadth in the region of the ovary and 1 4 mm. in that of the anterior testis. The anterior end is bluntly pointed and the posterior somewhat rounded. The anterior two-third of the body is covered with small backwardly pointed spines, which are numerous near the anterior end and which gradually decrease in number from before backwards till they disappear completely near the hinder end of the vitellaria The oral sucker is slightly larger than the ventral sucker measuring 0 28 mm in diameter, but in the immature specimen it is double the size of the ventral sucker. The ventral sucker measures 0·27 mm and is situated 2 0-2 6 mm distance behind the anterior end, *i.e.*, at the end of the first one-quarter body-length. The genital opening lies slightly to the left, 0·44 mm distance in front of the ventral sucker

The pre-pharynx is absent, the pharynx is globular measuring 0·225 mm. in diameter. The œsophagus is short, 0 2 mm in length and bifurcates almost behind the pharynx into the wider intestinal cæca which terminate a little in

front of the posterior end The cæca are somewhat swollen at their ends Their wall is composed of a single layer of columnar epithelium surrounded by a layer of circular and longitudinal muscle fibres. In the immature specimen the pre-pharynx is absent and the œsophagus is longer than that in the mature specimens

The testes lie in tandem near the posterior end of the body and have a deep notch at their posterior margin which gives them the appearance of a mammalian kidney. The posterior testis lies about 1 mm distance in front of the hinder end and 0·1 mm distance behind the anterior testis Both testes are broader than long and of almost equal size measuring 0·8 mm in breadth The vasa efferentia which could only be traced in sections arise as narrow ducts from the anterior surface of the testis. The vas deferens after entering the cirrus sac swells up to form a coiled thin-walled vesicula seminalis. The cirrus sac, 1 mm long and 0·3 mm broad at its posterior end, is crescent-shaped and has thick walls composed of longitudinal muscle fibres It is situated to the right side of the ventral sucker and partly overlaps it, extending behind as far as the ovary The vesicula seminalis as usual is filled with sperms and occupies nearly one-third length of the cirrus sac Its terminal tubular part which may be called the duct of the vesicula seminalis consists of two parts, a proximal narrow muscular tube of 0 05 mm length and 0 007 mm breadth, and distal broader part 0 22 mm long and 0 022 mm. broad ; the latter is somewhat coiled and opens by a valvular opening into the pars-prostatica. This duct with its valvular opening probably controls the passage of sperms from the vesicula seminalis into the pars-prostatica. The pars-prostatica is tubular, measuring 0 35 mm. in length and 0 022 mm in breadth, and is surrounded by the usual type of the prostate gland cells. The cirrus is small, muscular, and knob-like.

The rounded ovary, 0·12 mm. in diameter is situated immediately behind the cirrus sac, usually touching the right intestinal cæcum, at 0·37 mm distance behind the ventral sucker A short narrow ciliated oviduct, 0 05 mm. in length and 0 012 mm in breadth arises from the inner margin of the ovary near its posterior end and joins the small rather inconspicuous thick-walled duct of the receptaculum-seminis to form the ootype, where also the Laurer's canal joins from the opposite side The receptaculum-seminis is thin-walled and rounded, measuring 0·12 mm. in diameter It lies dorsally in the body and is always filled with sperms The Laurer's canal, 0·175 mm. long and 0·025 mm. broad, is a thick-walled ciliated duct which runs posteriorly to open to the exterior in the mid-dorsal line by a minute pore situated close behind the shell-gland-mass. Soon after the junction of the Laurer's canal with the oviduct the ootype turns ventrally to receive a small duct from the yolk reservoir and then becomes surrounded by the shell-gland-cells before passing into the uterus. The shell-gland-cells of the usual type are radially arranged around the ootype into which they open by their long narrow fibrillar ductules. The uterus arises as a narrow tube which turns towards the right side coiling spirally to form the right descending uterine coils. The descending uterus after reaching the anterior testis turns towards the left side to form the similarly coiled ascending uterus, which runs forward close inside the left intestinal cæcum. Its terminal end becomes less coiled and joins near the ventral sucker a short muscular metraterm of 0·8 mm. length, which opens into the common genital atrium in front of the opening of the cirrus sac

The vitellaria lie laterally outside the intestinal cæca both commencing the same level, 0 12 mm distance behind the ovary and terminating a short distance in front of the anterior testis, but not at the same level, the left

front of the posterior end The cæca are somewhat swollen at their ends Their wall is composed of a single layer of columnar epithelium surrounded by a layer of circular and longitudinal muscle fibres. In the immature specimen the pre-pharynx is absent and the œsophagus is longer than that in the mature specimens

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level with it as in *Cercorchis* *Paracercorchis* differs from *Telorchis* in the position of the testes, which in the latter genus lie midway between the ventral sucker and the hinder end. In *Telorchis* the genital pore lies to the left side midway between the ventral sucker and body margin, and the vitellaria extend behind and over the testes. *Paracercorchis* resembles the genus Telorchis only in the relatively small size of its cirrus sac It clearly follows from the foregoing points that *Paracercorchis* deserves the rank of a genus and though it combines in itself some of the characters of both the genera *Cercorchis* and *Telorchis*, it resembles the former more closely than the latter.

Diagnosis of the genus Paracercorchis—With the characters of the sub-family Body smooth or covered with spines Suckers of about equal size Genital aperture some distance in front of the ventral sucker slightly to the left side Testes strictly in tandem, at the posterior end of the body, rounded or broader than long and kidney-shaped with a notch on their posterior margin. Cirrus sac short extending a little distance behind the ventral sucker and situated to the right side. Vesicula seminalis coiled and joined by a duct to the long pars-prostatica. Cirrus small and knob-like. Ovary rounded situated in the anterior half of the body close behind the cirrus sac to the right side. Receptaculum seminis and Laurer's canal present Uterus inter-cæcal with descending and ascending uterine coils separated and regularly arranged in right and left halves of the body. Vitellaria laterally situated close outside the intestinal cæca, commencing behind the ovary and terminating a little in front of the testes.

REMARKS ON THE RELATIONSHIPS OF THE VARIOUS GENERA OF THE TELORCHIINAE

We give the following tree indicating the probable phylogeny of the genera of the sub-family Telorchiinæ. The

gland being always longer terminates more posteriorly. Each yolk gland consists of a large number of follicles, arranged in grape like bunches of twenty to thirty each. The lobes of the right, usually nine in number, are quite distinct, but those of the left one, about twelve in number, show a tendency to merge into each other. The longitudinal vitelline ducts lie in the narrow space between the intestinal cæca and the vitellaria and unite to form the transverse vitelline ducts in level with the second lobe of vitellaria The transverse vitelline ducts run obliquely forwards towards the mid-ventral line and unite to form a yolk reservoir from which a common vitelline duct runs anteriorly to join the ootype in the shell-gland-mass

The excretory bladder is typically Y-shaped The long main stem bifurcates behind the ovary into the two cornua, which receive the common collecting ducts and their branches from the body on each side The ova measure 0 0375 mm by 0 0175 mm in size.

SYSTEMATIC POSITION AND DIAGNOSIS OF PARACERCORCHIS

There is no doubt that *Paracercorchis* is closely related to *Cercorchis* and *Telorchis* which we recognise as separate genera, distinguished from each other by the size of the cirrus sac and the position of the genital pore, testes and the vitellaria. The genus *Paracercorchis* resembles *Cercorchis* in the vitelline glands restricted to the regions between the ventral sucker and the testes, larger number of follicle groups in the left vitelline gland, and the tandem position of the testes at the hinder end of the body but differs remarkably in the size of its cirrus sac and the metraterm (exceedingly long and coiled in *Cercorchis*), in the genital pore situated slightly to the left a short distance in front of the ventral sucker, and the vitellaria commencing behind the ovary and not in front of it or in

level with it as in *Cercorchis* *Paracercorchis* differs from *Telorchis* in the position of the testes, which in the latter genus lie midway between the ventral sucker and the hinder end. In *Telorchis* the genital pore lies to the left side midway between the ventral sucker and body margin, and the vitellaria extend behind and over the testes. *Paracercorchis* resembles the genus Telorchis only in the relatively small size of its cirrus sac It clearly follows from the foregoing points that *Paracercorchis* deserves the rank of a genus and though it combines in itself some of the characters of both the genera *Cercorchis* and *Telorchis*, it resembles the former more closely than the latter.

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REMARKS ON THE RELATIONSHIPS OF THE VARIOUS GENERA OF THE TELORCHIINAE

We give the following tree indicating the probable phylogeny of the genera of the sub-family Telorchiinæ The

genus *Dolichosaccus* in which we include *Lecithopyge rastellum* occupies the base of this tree on account of a number of primitive features such as (i) the great variability in the position of the genital pore which though it lies close in front of the ventral sucker in *Dolichosaccus rastellus* has wandered away in the other species of the genus so as to lie close behind the intestinal bifurcation. The genital pore in the other genera has become fixed either close in front of the ventral sucker as in *Cercorchis* or more in front as in *Paracercorchis*, or still further forward as in *Brachysaccus* or forward and more lateral as in *Protenes* (ii) The casual winding of the uterus into a few coils and its simple expansion during its course between the ovary and the testes in *Dolichosaccus* should also be regarded as a primitive feature. (iii) The enormous development of the vitellaria and the scattering of the vitelline follicles anywhere in the body that can provide sufficient space. (iv) Its habitat in the gut of Amphibian hosts and its distribution in Europe and Australia.

From *Dolichosaccus* we can derive the genera *Opisthioglyphe* and *Brachysaccus* which closely resemble it in many features In *Opisthioglyphe* the cirrus sac has shifted forwards so as to lie close in front of the ventral sucker except in one species, *i.e.*, *Opisthioglyphe locellus* in which it occupies a primitive position adjacent to the ventral sucker. The genital pore has also shifted forwards and occupies a more or less varying position in the different species. The main stem of the excretory bladder is short and bifurcates behind the testes into the two cornua of about the same length as the main stem which we consider a secondary condition characteristic of this genus only.

The genus *Brachysaccus* should be separated from *Opisthioglyphe*, in which Travassos has included it on account of the position of genital pore near the pharynx, shape

and position of the cirrus sac, the ovary being situated some distance behind the ventral sucker, the great development of the uterus, and the greater length of the main stem of the excretory bladder which bifurcates in front of the testes and not behind them *Brachysaccus juvenilis* Nicoll, which resembles *Opisthioglyphe ranæ* in the general shape and relative position of its organs probably forms an intermediate species between the two genera

We accept the genus *Cercolecithos* Perkins for the species *Cercorchis errectus* Molin It appears that this genus occupies an intermediate position between *Dolichosaccus* and *Telorchis* on account of the great development of the vitellaria and their extension behind and over the testes and the coiling of the uterus into distinct ascending and descending tracts, but it is more primitive than *Telorchis* on account of the position of its genital pore, which lies median immediately in front of the ventral sucker.

As pointed out before the genus *Paracercorchis* stands between *Cercorchis* and *Telorchis* *Protenes* should be considered as a specialised off-shoot from *Cercorchis* on account of the much forward and lateral position of its genital pore.

circular muscle fibres surrounded by a layer of longitudinal muscle fibres. The prostate gland cells form a large mass which almost fills the intervening space between the pars-prostatica and the cirrus sac. At about the middle of the length of the cirrus sac, the pars-prostatica passes into an extremely long and sinuous cirrus of 3·45 mm length, *i.e.*, about the same length as that of the former. The cirrus has thick muscular walls composed of an outer thick layer of longitudinal muscle fibres arranged in bands surrounding an inner layer of circular muscle fibres, it is entirely devoid of epithelium and is lined internally by the thick cuticle. In the retracted condition when it lies contained within the cirrus sac, it is produced into a number of narrow longitudinal outgrowths and is surrounded by a thick mass of fibrous parenchyma which fills the entirely intervening space between it and the cirrus sac.

The ovary is situated a little in front of the middle of the body to the right side in level with the basal end of the cirrus sac. It is spherical, but in the flattened specimens it appears transversely elongated presenting an ovalish outline, measuring 0·29 mm in length and 0·63 mm in breadth. The oviduct is ciliated. It arises from the middle of the posterior margin of the ovary, and after running dorsally towards the left for a short distance it turns towards the right side to join the Laurer's canal and the yolk reservoir. The receptaculum seminis is very small rather rudimentary representing the internal end of the Laurer's canal. The Laurer's canal is slightly coiled and ciliated, it opens to the exterior dorsally in the region of the ovary by a small pore lined with cuticle. The ootype is surrounded by the radially arranged shell-glands of the usual shape. The uterus is much coiled, overlapping the intestinal cæca and consisting of the right descending and the left ascending parts which are not easily distinguishable. It joins the long muscular metraterm of 5 mm length and 0·225 mm breadth

at about the level of the junction of the pars-prostatica with the cirrus The metraterm is coiled like the cirrus sac and is composed of a thick layer of longitudinal muscle fibres, surrounded by an equally thick layer of circolar muscle fibres. The ova are small measuring 0·0375 mm. in length and 0·0175 mm in breadth.

The vitellaria lie laterally near the body-wall overlapping dorsally and ventrally the intestinal cæca and the lateral extensions of the uterine coils They commence 1·8 mm. in front of the ovary, *i e*, about the middle of the distance between the latter and the ventral sucker, at about the junction of the uterus with the metraterm and terminate in front of the testes but not at the same level. The left yolk gland is longer and terminates always behind the right one Each vitelline gland is composed of a large number of follicles arranged in lobes of 30—50 each, which nearly run into each other to give the gland a band-shaped appearance. There are twelve such lobes in the left gland and nine in the right one The transverse vitelline ducts arise immediately behind the ovary and unite together to form a small vitelline reservoir which lies dorsally on the shell-gland-mass

The excretory bladder is Y-shaped, the long main stem bifurcates immediately behind the ovary into two cornua, which extend as far forwards as the ventral sucker. The excretory opening is situated at the hinder end of the body.

Diagnosis of C. dhongoki.—Body elongated, spinous, broadest anteriorly. Genital aperture immediately in front of the ventral sucker. Suckers equal in size, ventral sucker situated at the end of the first one-sixth body-length. Pre-pharynx and œsophagus absent; intestinal cæca terminating near the hinder end. Testes strictly in tandem at the hinder end; anterior testis slightly smaller than the posterior. Cirrus sac exceedingly

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long, tubular, and sinuous. Metraterm sinuous and exceedingly long, nearly of the same length as the cirrus. Ovary situated to the right side in level with the basal end of the cirrus sac Laurer's canal present, receptaculum seminis very small and rudimentary Vitellaria laterally situated near the body-wall overlapping the intestinal cæca, commencing behind the ventral sucker at about the middle of the distance between the latter and the ovary, and terminating in front of the testes but not at the same level, the right gland ending in front of the left Uterus extremely coiled overlapping the intestinal cæca. Excretory bladder Y-shaped, the main stem bifurcating immediately behind the ovary and the cornua extending as far as the ventral sucker.

Plate 1

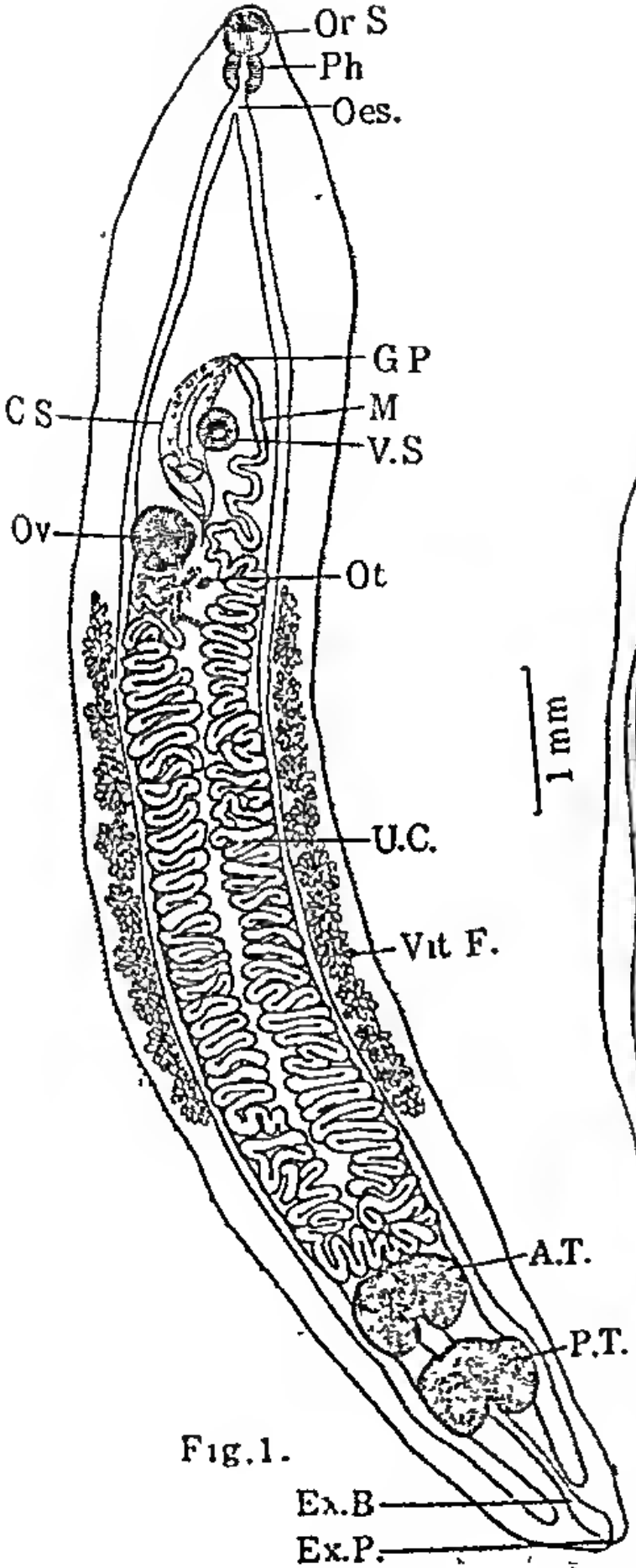


Fig.1.

Fig.2.

1 mm

1 mm

EXPLANATION OF PLATES I—III

Fig. 1.—Ventral view of adult *Parancercorchis pellucidus*
Fig. 2.—Ventral view of a young *Parancercorchis pellucidus*
Fig. 3.—Ventral view of an adult *Cercorchis dhongokii*
Fig. 4.—Ventral view of a young *Cercorchis dhongokii*
Fig. 5.—Diagrammatic view of male genetalia of *P. pellucidus*
Fig. 6.—Diagrammatic view of female genetalia of *P. pellucidus*.
Fig. 7.—Diagrammatic view of female genetalia of *C. dhongokii*
Fig. 8.—Transverse section passing through the region of Pars-prostatica of *C. dhongokii*
Fig. 9.—*Transverse section passing through the region of cirrus of C. dhongokii*

ABBREVIATIONS

A.T	Anterior testis	Ovd.	Oviduct
C	Cirrus	P. T	Posterior testis
C. S	Cirrus sac	Par	Pars-prostatica.
Com. Vit	Common vitelline duct	Ph	Pharynx.
Cu	Cuticle.	Pr G	Prostate gland
Ex B	Excretory bladder	Pre	Prepharynx.
Ex P	Excretory pore	R. S.	Receptaculum seminis
G. P	Genital pore.	S	Spines
In. C	Intestinal caecum	Sh. G	Shell gland.
L. C.	Laurer's canal.	U.	Uterus
M	Metraterm	U. C.	Uterine coils.
O	Ova	V. S.	Ventral sucker.
Oes.	Oesophagus.	Ves S	Vesicula seminalis
Or S	Oral sucker.	Vit. F.	Vitelline follicle
Or	Ootype	Y. R	Yolk reservoir.
Ov.	Ovary.		

Plate 2

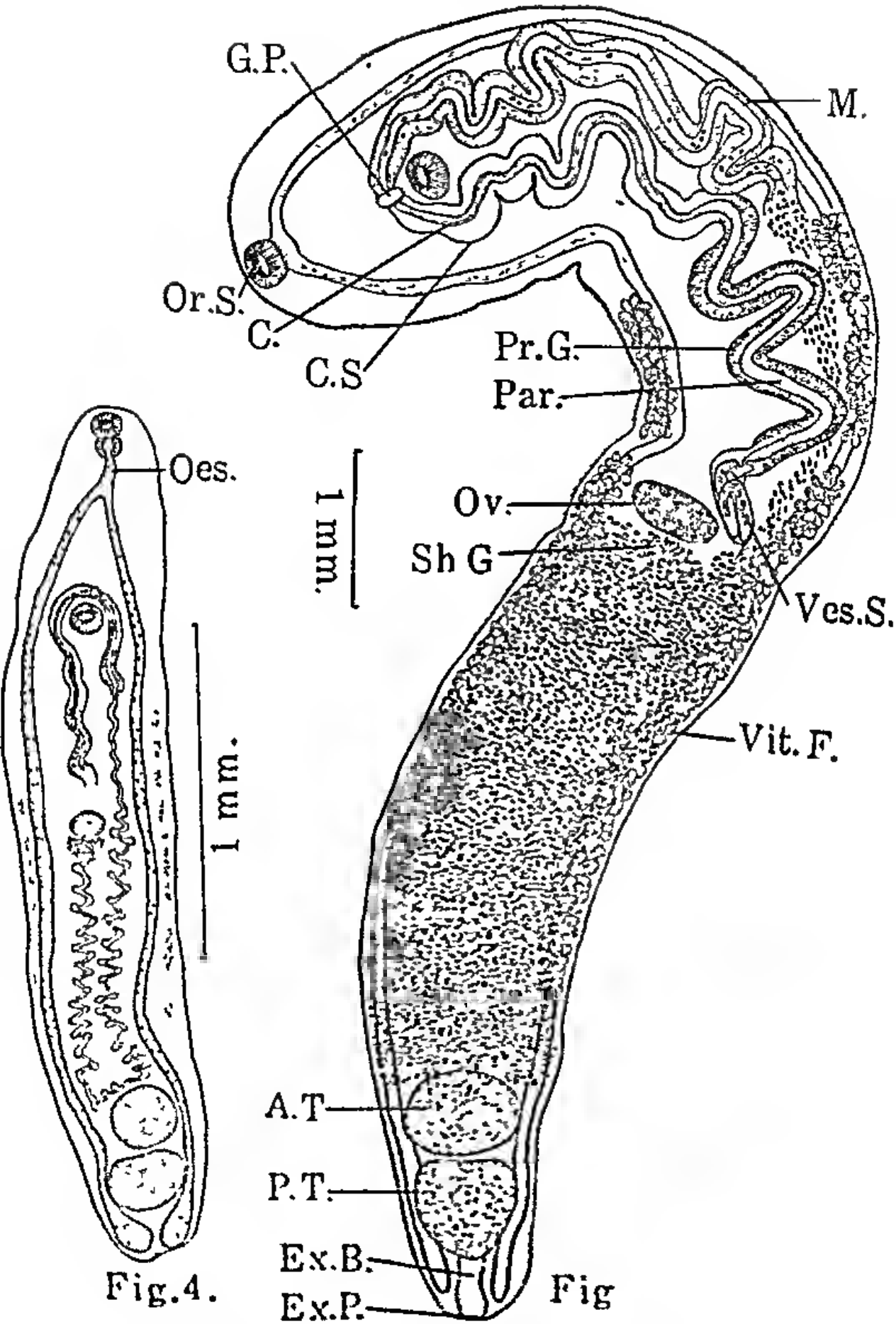


Fig.4.

Fig

Plate 3

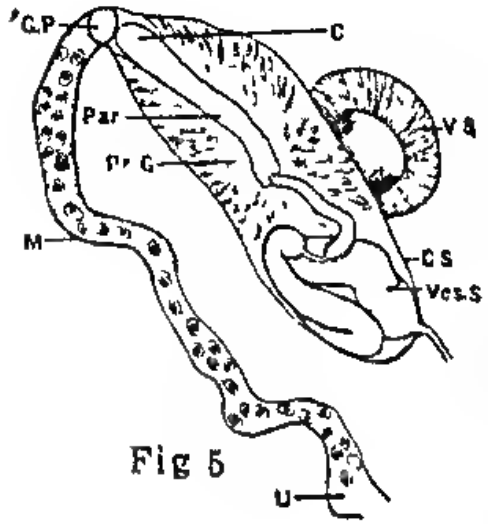


Fig 5

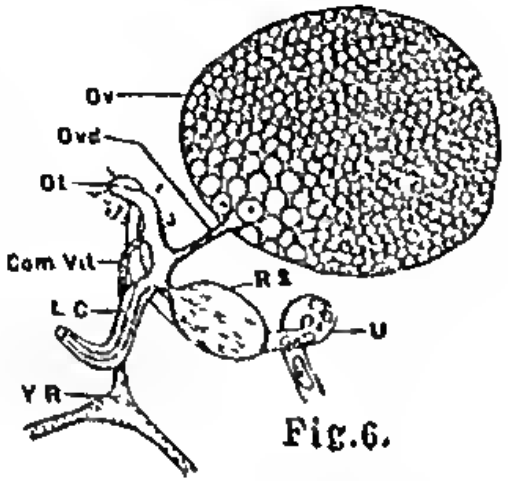
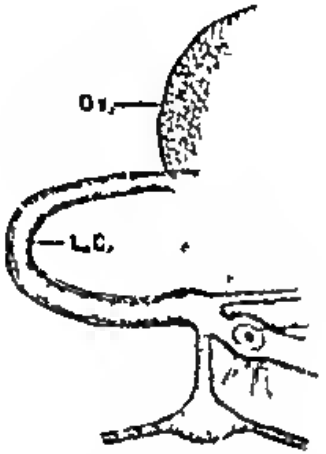


Fig.6.



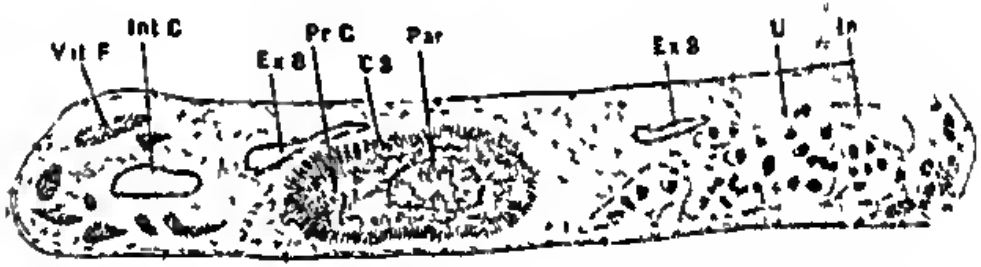


Fig.8.

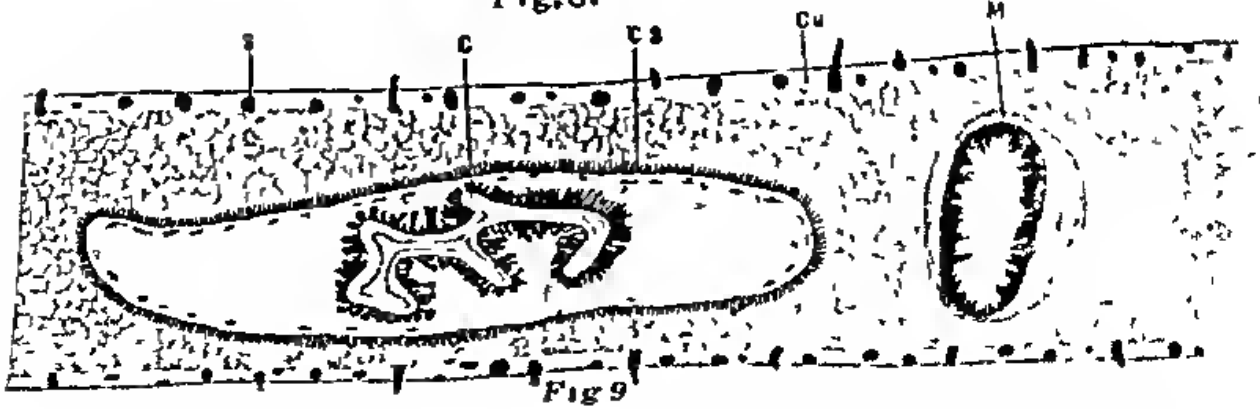


Fig 9

ON CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF SCYLLA SERRATA (FORSK)

BY

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INTRODUCTION

Recent work on the oogenesis of various animals has shown that the part played by the various cytoplasmic inclusions varies in nature considerably in different organisms So far as the Crustacea are concerned, the only recent contributions are those of Harvey on the Plymouth shore crab, Carcinus maenas, and of King on a primitive Isopod, Oniscus. The Indian crustaceans have lain totally unexplored, and there is, therefore, enough justification for undertaking the present piece of work.

In Oniscus, King (42) has found that the Golgi elements form the fatty yolk and that there are no nucleolar extrusions nor is there any area which may be considered to be homologous with the "Yolk nucleus of Balbiani" Proteid yolk, on the other hand, was observed to have been formed in relation to mitochondria. A peri-nuclear zone of mitochondria has been noted and after the formation of this zone the mitochondria are said to swell up and give rise to proteid yolk.

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Harvey (36) worked out the cytoplasmic inclusions with special reference to yolk-formation in the oogenesis of Carcinus and has recorded interesting results In the younger oocytes he could not discern the "Yolk nucleus of Balbiani' area He has come to the conclusion that "fatty yolk is formed independently in the cytoplasm and albuminous yolk is produced in relation to Golgi bodies and probably also mitochondria."

The results noted above suggested a further examination with a view to throw more light on the method of yolk-formation in crabs This work was also taken up by Nath and his collaborators at Lahore but due to paucity of material no conclusive results could be recorded.

MATERIAL AND TECHNIQUE

The specimens were collected near the delta of the river Ganges in Calcutta at fortnightly intervals from the month of September onwards The female specimens collected for this work could easily be identified and distinguished from the male ones by the fact that they are of smaller size and possess a broad abdomen loosely attached on the ventral side of the thorax. The male specimens, on the other hand, are larger in size and each possesses a conical abdomen and clasping organs

In the month of September, female specimens of varying sizes were dissected but the ovary was so feebly developed in this season that even in the largest specimens nothing but a mass of germinal epithelial cells could be obtained in the form of a delicate transparent gland-like tissue This tissue lying just beneath the dorsal carapace in the form of two coiled strings across the hepato-pancreas, develops gradually into a thicker ramifying mass in the specimens obtained during the months of October and November. In the early period of the month of October

the ovary is fully packed with just differentiated oogonial stages and a few young oocytes By the end of the month of October and onwards till December we could find oocytes of various stages Thus the most fruitful results were obtained from the experiments carried on from the month of October till the end of January The ovary during this period begins to become mature and in larger specimens presents a fully matured reddish pulpy mass occupying a large area

Small pieces of the ovary were fixed in various kinds of fixatives. The duration between the killing of the animal and the fixing of the ovary did not exceed a couple of minutes and thus all the possibilities of post-mortem changes in the tissue were minimised.

For the demonstration of the Golgi apparatus, the methods used were Da-Fano's Cobalt nitrate method, Cajal's uranium technique and Ludford's latest modification of the Mann-Kopsch fixative. The best results were, however, obtained by the techniques recommended by Da-Fano and Ludford.

Ludford's method proved to be most satisfactory for the demonstration of the Golgi apparatus. The material was fixed in Ludford's fluid (equal parts of saturated corrosive sublimate in salt solution and 1 per cent osmic acid) for eighteen hours and after being washed thoroughly for an hour with distilled water to remove every trace of corrosive sublimate, it was kept for three days in 2 per cent osmic acid at 35°-40°C. The sections were cut 5μ in thickness and the subsequent bleaching was effected by Hoaneguy's process, by treating the sections with 1 per cent aqueous solution of potassium permanganate for 5-10 seconds and then with 4 per cent solution of Oxalic acid for 1-2 minutes.

The slides were stained in Altmann's acid fuchsin, toluidene blue and aurantia.

In Da-Fano's Cobalt nitrate method the material was fixed for 20 hours at 20°-25° C and then kept in a 2 per cent

solution of silver nitrate for 48 hours in order to effect proper impregnation The extra silver was reduced by the methods indicated in the Vade-Mecum (27). Sections 6μ in thickness were cut and the slides were toned by 2 per cent gold chloride and 5 per cent hypo solution The sections were stained either with safranine and light green or with iron alum and haematoxylin

For mitochondria the best fixatives were found to be Champy-Nassonov's chrome-osmium technique, Regaud and Regaud-Tupa's bichromate methods The material, after fixation in Champy's fluid, was put in 2 per cent osmic acid for post-osmication at 35°C for 5-7 days. Post-chromatisation was necessary in case of Regaud's fixative for 2-3 weeks.

All the osmic and dichromate preparations were stained by Champy-Kull method, *e g*, acid fuchsin, toluidene blue and aurantia.

For nucleolar extrusions, Bouin's picro-formol-acetic acid fixative was used which dissolves out cytoplasmic inclusions like Golgi bodies and mitochondria but fixes the nucleus and its derivatives The sections were stained in Mann's methyl-blue-eosine and satisfactory results were obtained.

Pure turpentine free from all traces of acid was used in order to dissolve out all free fat if present. The *proteid* yolk bodies which appear as greyish brown bodies in osmic acid do not get dissolved in turpentine even after prolonged treatment, whereas, the fatty yolk bodies readily disappear being reduced by turpentine, leaving clear vacuolar areas each surrounded by an osmiophilic rim.

Centrifuge experiments were also carried out in a dark room during the winter months The material was kept in the centrifuge which was rotated at the rate of 3,000 revolutions per minute This operation was continued for three hours and the material was taken out speedily, and fixed as usual

INTRA-VITAM EXAMINATION

Intravitam examination of tissues has been tried by Parat, Gatenby, Möllendorff and others in order to demonstrate the disposition and behaviour of the various cytoplasmic inclusions in the fresh material Various vital dyes, *e.g*, Neutral red, Janos green B, Methylene blue, Nile blue, Trypan blue, etc, have been recommended to stain the various inclusions in the fresh material Parat made use of Neutral red and Janus green B in very dilute concentrations to stain the Golgi bodies and mitochondria respectively.

The stock solution of the Neutral red and Janus green B was made according to Bhattacharya and Das's formula, *i e.*, by dissolving 1 gm of fresh dye in 50 c c. of 6/1000 salt solution. The stock solution in bottles was then placed in an incubator at 38°-40°C for 24 hours This solution is diluted to bring about a dilution of 1/25000

Pieces of ovary were kept in this pink solution for about an hour and examined from time to time under oil immersion lens

Besides these dyes 2 per cent osmic acid was also used as recommended by Gatenby, Bhattacharya, Nath and others for the examination of the yolk bodies The fresh material was placed in 2 per cent osmic acid for about half an hour and then examined from time to time to note the effects of osmic acid on cell organs.

OBSERVATIONS

Golgi Apparatus

In Da-Fano preparations stained with Safranine and Light green, the Golgi elements appear as black bodies, some spherical in shape with a chromophilic rim and a chromophobic centre, and others representing a semilunar appearance—the Golgi crescents or dictyosomes. In an advanced oogonium

(Fig 1, Pl 1) which has apparently been differentiated out from one of the germinal epithelial cells, a few very small isolated black bodies (Gb) are visible in the clear cytoplasm During this stage the cytoplasm presents a very clear and homogeneous area surrounding the nucleus (N), which itself is of a denser structure containing many nucleoli embedded in a sort of reticulum. For the sake of comparison and identification similar preparations of silver and osmic methods were examined and the results verified and confirmed. In the early oocytes the differentiation of the Golgi elements into a chromophilic rim and a chromophobic area is not well marked because of their being of extremely minute size, but in the older oocytes they appear as dictyosomes and vacuoles with a dark chromophilic rim and a clear chromophobic area. In the early stages the nucleus occupies the major portion of the oogonium surrounded by a thin area of marginal cytoplasm There are many nucleoli within the nucleus at this stage and from the beginning they have a tendency to shift themselves to the periphery of the nucleus

Figs. 2, 3, 8, 9 (Pls 1, 2) represent the early oocyte stages Here the cytoplasm occupies a larger area surrounding the nucleus The Golgi elements (Gb) increase in number and they lie closer to each other on one side of the nucleus With the growth of the oocyte (Fig 10, Pl. 2), the Golgi elements lying closer to each other accumulate in mass-formation in a juxta-nuclear position.

Figs. 4 and 11 (Pls. 1, 2, Y Nuc) show the formation of the area called the "Yolk-nucleus of Balbiani." The Golgi bodies (Gb) acquire the usual complex form as a compact massive structure situated adjacent to the nucleus, *i.e.*, in a juxta-nuclear position The "Yolk-nucleus of Balbiani" has been described by Munson (50), D' Hollander (16) and others This yolk-nucleus is the homologue of the archoplasmic area as recorded by Gatenby in his series of papers in the Q J.M.S and by Ludford (45) in Patella It

is a focus of growth and dispersal so for as Golgi bodies are concerned and takes in stain readily, thus standing out in sharp contrast to the cytoplasm of the egg in general. At this stage we can notice easily the differentiation of Golgi elements into two kinds, (1) those that are spherical, (2) those that ore crescent-shaped Both possess a dark chromophilic rim and o clear chromophobic core In close association with Golgi elements some bigger bodies are visible which ore totally blackened with the reaction of the osmic acid probably dne to their being fatty in nature These bodies bave been identified ae fatty yolk bodies

For some time this yolk-nncleus of Balbiani persists on ono sido of the nucleus bnt gradnally tho Golgi vesicles and dictyosomos begin to get detached from this compact massand migroto into the general cytoplasm. (Figs 5, 12, 13, Pls 1,2) Tho compact area can still be distinguished from tbe rest of tho granular area (Fig. 13, Pl. 2). In a full-grown oocyte (Figs. 6, 14, Pls. 1 ond 2), tho yolk-nucleus is completely disorganised and the Golgi elements (Gb) are found ecatterod througbout tho cytoplasm. Tho fatty yolk bodies (Fy) lying either independently or in closo association with tho Golgi elements are to be seen quito distinctly. In Lndford preparations some fairly largo greyish brown spheres are visible. They are tho olbnminons yolk spheres (Ay). No apparent relationslup has been observed between the Golgi elements and the formation of nlbuminous yolk

Mitochonndia

The best results were ohtained from Regoud's formol-biehromate method followed by a prolonged ehromatisation for 2-3 weeks, and from Champy-Nossonov's techniques. Mitochondria are also visiblo in Regaud-Tupa preparations. Dichromate tcehniques are cvelnsively meant for the demonstration of mitochondria whereas Champy's fluid may fix the Golgi elements and the associated yolk as well.

In the oogonial stages the mitochondrial granules or chondriome (M) (Fig 15 Pl. 3) are observable in the form of a few dusty particles stained faintly with acid fuchsin In the early oocytes (Figs 16, 17, Pl 3) the mitochondria come closer together adjacent to the nucleus and form, alike Golgi elements, a complex juxta-nuclear cap-like investment, the so-called "Yolk-nucleus of Balbiani", (Y.-Nuc) (Figs. 18 and 25, Pl 3) This Yolk-nucleus area, in our opinion, functions as a centre of growth and dispersal for both Golgi bodies and mitochondria. This area may be regarded as the seat of intense cytoplasmic activity at a particular stage of development

This heavily stained area gradually enlarges so as to surround the nucleus forming a perinuclear zone containing dusty mitochondria stained pink in acid fuchsin (Fig 21, Pl 3) Figs 23 and 27 (Pl. 3) represent fully developed oocytes where the mitochondrial granules have dispersed throughout the cytoplasm, and amongst these mitochondrial granules are found some big spheres stained cherry-red with acid fuchsin. These are the albuminous yolk bodies (Ay) These bodies are visible even in very early oocytes

In spite of keeping the material for post-chromatisation after Regaud's bichromate technique (Ballard's method—10a) for a period of *more than a month*, we *observed* no filamenter structures which could be identified as mitochondria

It is a noteworthy fact that mitochondria remain very fine and dusty and the granules have not been observed at any stage to swell up or enlarge or take any part in the formation of yolk bodies

Nucleolar Extrusions

The material was fixed in Bouin's picroformol-acetic acid fluid, so that the two important inclusions, the mitochondria and Golgi bodies were dissolved out. The staining

wae done by Mann'e methyl-blue-eosin and very clear nucleolar extrusions were noticed lying outside the nucleus in a similar way as observed by Nath in Lithobius, Crossopriza, Scorpions, etc., Ludford in Patella, Gresson in Tenthredinidae, Harvey in Carcinus, and Gatenby in Saccocirrus.

Fig 28 (Pl. 4) represents an early oocyte. The nucleus (N) is a large ovoid body occupying a large space in the ooeyte, containing many small rounded nucleoli (Nu), basophilic in consistency (staining deep purple with Mann's methyl-blue-eosin and deep blue with haematoxylin). These nucleoli are embedded in the reticulum of the nucleolymph and the cytoplasm presents a clear, homogeneone area In later stages (Figs 29, 30, Pl 4), one of the nucleoli growe in size and is converted into a prominent baeophil nucleolne (B. Nu), whereas the other nucleoli have a tendenoy to move towarde the periphory and plaster themselves around the nuclear membraae which looks like a beaded ring As the oocyte growe in size (Fig 30, Pl 4), the peripheral nucleoli have a tendency to come out of the nuclear membrane into the cytoplasm in the form of granular extrusions. In the meantime the basophil nucleolus becomes a very prominent body It becomes larger and buds off deeply staining basophil bodies which pass out into the cytoplasm through the nuclear membrane (Figs. 29, 30, 31, Pl 4). Figs. 29 and 30 represent oocytes in which a few granules budded off by the nucleolus, come out of the nuclear membrane, while others are still sticking to the membrane Fig 31 (Pl. 4) represents a later stage when the nucleolar extrusions have dispersed fairly evenly throughout the cytoplasm It is to be noticed in these cases that the nucleolar omissions (N.E) scattered in the cytoplasm are stained lightly with Mann's methyl-blue-eosin and are basophilic in the beginning, but become acidophil bodies when scattered in the cytoplasm and ultimately disorganize due to fragmentation

This change in the behaviour of the nucleolar extrusions may be noticed easily by the staining reactions of Mann's methyl-blue-eosin Probably the nucleolus during the period of its marked activity transforms itself into an amphophil body (A Nu) containing round basophil bodies inside a lighter acidophil ground substance as observed by Nath in Buthus judaicus (57)

It is remarkable that the basophil nucleolus which persists even in older oocytes, occasionally, has a tendency to come out of the nuclear membrane as a whole into the cytoplasm (Fig. 37, Pl 5) But, during this process no rupture of the nuclear wall has been observed Probably in the cytoplasm also, it buds off some granular extrusions as observed by Nath (57) in Euscorpius napoli and Buthus judaicus This shifting of the nucleolus as a whole from the nucleolymph to the cytoplasm (Figs 37 and 38, Pl 5) has been recorded by Nath (57) in scorpions and by Hennegny in vertebrates These nucleolar extrusions have not been observed, however, at any stage to be directly metamorphosed into albuminous yolk spheres but probably they bear their influence in some way towards yolk-formation

The Formation of Yolk Bodies

Two kinds of yolk bodies are easily distinguishable—the fatty yolk, and the albuminous yolk. The fatty yolk appears to arise through the intervention of Golgi bodies directly In Da-Fano preparations there is very little possibility of the fat being fixed and the fatty yolk bodies appear as clear vacuolar spheres each in association with a Golgi element. Figs. 32, 33 and 34 (Pl 4) represent oocytes at various stages of development showing the method of fatty yolk-formation At the early stages of development, inside and around the yolk nucleus (Fig 32, Pl 4)

some larger vacuolar bodies begin to appear amongst the scattered Golgi vesicles and dictyasomes.

In Ludford and Champy preparations also (Fig. 14, Pl 2 and Fig. 35, Pl 4) in close association with Golgi elements, larger spheres are visible which, being fatty in nature, appear as solid dull black bodies and do not show any sharply distinguishable hemiaphilic rim or crescent. These spheres are fatty yolk bodies (Fy). To confirm their fatty nature, sections were treated with pure turpentine for varying periods and then examined under an oil-immersion lens It was noticed that the original solid dull black bodies were totally decolourised leaving clear vacuolar areas either attached to an osmiophilic crescent or surrounded by a black rim, thereby proving their fatty constituency. The intermediate stages between the transforming Golgi bodies to fatty yolk spheres are also found. The Golgi elements which are non-fatty in the beginning, swell up and the fat is deposited within their chromophobic area (archoplasmic area). Thus there seems little doubt that these fatty yolk bodies are formed directly by the Golgi elements In a well developed oocyte, these fatty yolk discs so formed by the metamorphosis of the Golgi elements are seen scattered throughout the cytoplasm

Albuminous or protoid yolk has been described by different cytologists to have originated under the influence of either nucleolar extrusions or mitochondria, and sometimes, *de novo*, in the cytoplasm and rarely under the influence of Golgi bodies.

Nath (53) and Ludford (45) have observed in many invertebrates that nucleolar material from the nucleus comes out in the cytoplasm and contributes directly towards the formation of deutoplasmic inclusions or vitellogenesis. In early oocytes of Champy and Regaud preparations, occasionally, we are able to notice some large bodies which appear as greyish brown spheres in osmic preparations and

Small pieces of the ovary were placed in a trough containing a dilute pink solution of Neutral red dye (1/25,000) dilution for 20-45 minutes The ovary was then teased out gently and examined from time to time in a dark room under oil-immersion lens in artificial light (1000 candle power) Some of the young oocytes (Fig 39, Pl 5), when examined carefully, were observed to contain a nucleus and a thick granular mass juxta-nuclear in position (Y Nuc.) which we identify as the "Yolk-nucleus of Balbiani' which takes the same place in the fixed preparations topographically

In more advanced oocytes (Figs. 40 and 41 Pl 5), the Golgi bodies appear scattered in the general cytoplasm as discrete bodies, some vesicular with a chromophilic rim surrounding a chromophobic centre and others as crescent-shaped dictyosomes associated with an archoplasmic area We get exactly similar bodies in fixed Da-Fano and Ludford preparations (Figs. 6 and 14, Pls 1 and 2). In close association with these Golgi elements we find some large highly refractive vacuoles with a surrounding rim or a dictyosome These are fatty yolk-spheres as ascertained by the treatment of the material with 2 per cent osmic acid.

Besides the above two types of structures, dispersed in between the Golgi elements, groups of very small vacuolar structures have been observed which take a cherry-red colour with the dilute pink solution of neutral red These bodies make their appearance after the material has been in dilute neutral red dye for about 30-45 minutes These have been identified as "Vacuome" by Gatenby (26), Bhattacharya (6), and Das (15) in other animals There is apparently some relationship between the Golgi elements and the vacuome as it has been observed that round about patches of vacuome, some black dictyosomes or Golgi vesicles are situated (Figs. 40 and 41, Pl 5)

In a well-advanced oocyte (Fig 41, Pl. 5) (Vc) as many as four or five patches of "Vacuome" have been observed Nothing definite has yet been known regarding the function and behaviour of the "Vacuome" but it has to be admitted that it is a cell structnre revealed only in fresh material. Fig 42 represents an oocyte eeen after treating the material with 2 per cent osmic acid fnr abont 10 minutes and Fig 43 represents another oocyte nf abont the same size examined after half an hour. The Golgi badies appear as discrete heterogeneous elements, nnn-fatty in nature as proved by their remaiuing as refringent bodies (Gb) They are easily made out. In association with them somn swollen Golgi bodies havo been found, of a fatty nature, due to the deposition of freo fat inside tho chromophobic or archoplasmic area Fig. 43 shows that tbo oocyte after having been treated for half an hour in 2 per ceut osmic acid brings prominently into view swollen up Golgi elemeuts which appear as darkor spheres The gradnal etagee between the developing Golgi elements and the formation of fatty yolk spheres are clearly noticed and it may evidently bo concluded that the Golgi elemeats swell up and giro riso to fatty yolk directly.

DISCUSSION

Gnlgi Apparatus

In considering the rôln of the Golgi apparatus in oogenesis we have to take intn cnnsideratioa all that has been discovered concerning its behavinnr in the cell In spito of the various differences in the form and behaviour of Golgi apparatus and nitochondria as described by Nath (55), Bhattacharya (4), Gatenby (21, 26), Parat (65, 67), Ludford (45), Weigl (78), and others, there are some common characteristics found in them. They are capible of independent movement within tho cell. They grow by assimilating the

necessary food substances from the cytoplasm and increase in number probably by fission

The exact behaviour of the Golgi apparatus as well as that of mitochondria during oogenesis, differs in detail in most cases that have been investigated. In the germ cells of the vertebrates and invertebrates the apparatus consists of separate rods, crescents, rings and sometimes granules These are revealed by silver and osmic techniques The differentiation of Golgi elements into a chromophilic rim and chromophobic area is almost common to all germ cells of vertebrates and invertebrates

In the animal under investigation the best results to demonstrate Golgi apparatus were obtained by Ludford and Da-Fano techniques The impregnation obtained in the case of Da-Fano after keeping the material in 2 per cent silver nitrate for 48 hours was specific, unlike the observation of Harvey who fixed the material for 4-6 hours only.

In a well-developed oocyte the Golgi elements exist in two forms The spherical vesicular Golgi elements with a chromophilic rim and chromophobic centre and the semilunar forms or dictyosomes enclosing a portion of archoplasm

Parat (65, 67) has recently emphasised that the Golgi elements and the vacuome are homologous His conclusion is based on the assumption that the neutral red staining vacuome are Golgi bodies whereas the associated chromophilic substance is either an artifact or constitutes a special kind of mitochondria—the so-called Lepidosome.

Recently, Bowen (9) in plant cells, and Gatenby (25) in male germ cells have noticed the two above-mentioned structures lying separately. Gatenby observed that the so-called Golgi bodies of Parat are really vacuolar structures associated with crescent-shaped bodies—the dictyosomes Gatenby, further describes the vacuome as an aggregation of vacuolar structures which are supposed to have been produced by the chromophilic rim of the Golgi elements So,

the real substance of the Golgi element is constituted by the chromophilic rim (the dictyosome) and not by the associated vacuole—Parat's Golgi body.

In the youngest oocyte the Golgi apparatus lies in a diffused system consisting of a few granules which stain black with silver or osmic acid The Golgi elements in a later stage form a compact mass, juxta-nuclear in position, the so-called "Yolk-nucleus of Balbiani." This structure has been described by various authors (50, 3, 15, 10) as the centre of growth and dispersal of Golgi elements. At this stage when the yolk-nucleus of Balbiani or the idiosome area (Bowen) is established, the Golgi bodies appear as discrete spherical and crescent-shaped dictyosomes.

Harvey in Carcinus (36), has failed to discern the formation of yolk-nucleus He says, "Golgi elements increase in number eventually without any diminution in size and at this period a marked peripheral concentration of the Golgi elements becomes apparent." Again he adds, "As the yolk increases the yolk droplets occupy the outermost regions of the cell, until the majority of the Golgi elements are eventually crowded into the narrow perinuclear area . . ." In the animal under examination, no such perinuclear concentration of the Golgi bodies has been observed and also no relationship could be established between the yolk bodies (proteid yolk) and the Golgi elements. Golgi bodies have been observed to play an important part in the formation of fatty yolk, unlike the observation of Harvey (36) in Carcinus, where the fatty yolk is said to be formed from the cytoplasm independently and without the aid of any of the cytoplasmic inclusions. The formation of the proteid yolk by Golgi elements as observed by Harvey, in Carcinus, must be an interesting feature, because of its rare occurrence.

Mitochondria

In the oogonial stages mitochondrial granules are visible with great difficulty. In the early oocytes the mitochondrial granules, alike Golgi elements, occur in the "Yolk-nucleus of Balbiani" Harvey, in Carcinus, found "a slight concentration of mitochondria effected in immediate neighbourhood of nucleus," but he ascribes this concentration to the absence of a large number of mitochondria Later on, he observed a perinuclear zone of mitochondria Probably Harvey's slight concentration of the mitochondria in the immediate neighbourhood of the nucleus is the "Yolk-nucleus of Balbiani" as described in this animal. Harvey might have missed the stages of the formation of the yolk-nucleus and therefore took into consideration only the perinuclear zone of mitochondria

It is a remarkable fact that during the oogenesis of this animal the mitochondrial granules always remain dusty and granular They have never been observed to increase much in size In spite of very careful search these bodies have not been observed to take any part in the formation of any reserved food substances in the oocytes Many authors have ascribed to mitochondria the formation of proteid yolk either directly or indirectly King, in Oniscus (42), records the proteid yolk as being directly formed by the swelling up of the mitochondrial granules In Carcinus, Harvey has observed, "the albuminous yolk arises in the cytoplasm under the influence of Golgi bodies and probably mitochondria." But a careful search in this animal, has not revealed any relationship between mitochondria and albuminous yolk-formation

During recent years many cytologists have been able to discover the filamenter mitochondria in the oocytes of many animals, *e g*, King (41), Hibbard (38), Das (15), Bullard (10a), and others In spite of post-chromatization

of the material for more than six weeks no filamenter mitochondria could be observed in our material.

We are inclined thus to conclude that mitochondria plays a rather insignificant part in the oogenesis of this animal

Nucleolar Extrusions

Recently, Harvey in Carcinus (36), has observed a process of nucleolar budding and the "probable emissions of nucleolar substance" from the nucleus to the cytoplasm. We have tried to substantiate the above conclusion by carefully working out the nucleolar behaviour during the oogenesis of this animal Alike the observations of Harvey, we find, there are many nucleoli in the beginning but in later stages of development one nucleolus becomes prominent and gives out the extrusions. In this animal it has been definitely observed that the nucleolus in the beginning is a basophilic structure which afterwards turns oxyphilic

The change in the staining reactions of the basophilic nucleolus into oxyphilic bodies in the cytoplasm has been observed by Nath in Culex (59), and more recently in Spider (58), and Scorpions (57) In Euscorpius napoli and Buthus judaicus, there is copious discharge of prominent round and deeply staining basophil bodies from the nucleus into the cytoplasm of the egg. "They are first basophil and later become acidophil and ultimately disappear as whole bodies." Gresson (31, 32), working on the oogenesis of sawflies (Tenthredinidae), has observed that in the early oocytes of Thrioax imcula, the nucleoli are basophilic. As the oocytes increase in size the nucleoli develop an oxyphilic margin, which later on become rounded off and separate *from* the basophilic body. The basophil nucleolus buds off a number of basophilic extrusions which remain embedded in the nucleolymph and have not been observed to pass out in the cytoplasm. The oxyphilic part in

the meantime undergoes a period of activity and numerous oxyphil buds are liberated which migrate towards the nuclear membrane and eventually pass out into the cytoplasm

Ludford in Patella (45, 46), has also observed a remarkable differentiation of the nucleolus into an oxyphil and basophil part He suggests that the oxyphil nucleolus of the early oocytes gives rise to a basophil portion and then they gradually separate till both of them bud off extrusions of both kinds But in ooplasm only oxyphil bodies have been observed whereas the basophil ones remain within the nucleus

Wilson points out that the staining reactions of the nucleoli often vary materially at different periods in the history of the nucleus so that the same nucleolus may be at one time oxyphilic and at another time basophilic

In our material it has been observed that the staining reactions of the nucleolus and nucleolar extrusions change from basophilic to oxyphilic during their passage from the nucleus to the cytoplasm Occasionally, it has been observed in this animal, that the nucleolus as a whole or a major part of it comes out from the nucleus to the cytoplasm apparently without injuring the nuclear wall This is, no doubt, an interesting phenomenon and has also been observed by Nath (57) in Scorpions No sooner, it lies in the ooplasm, than the staining reactions are reversed and an oxyphilic structure instead of a basophilic one is noticed Frequently, this oxyphil body seems to bud off oxyphil extrusions in the cytoplasm.

Bhattacharya (3), Nath (53), Gatenby (22), Ludford (46) and others working on vertebrates and invertebrates have laid stress upon the phenomena of nucleolar extrusions and in certain cases have attributed to the nucleolar extrusions the origin of albuminous yolk It may, therefore, be said with a fair amount of certainty, that in many animals the nucleolar extrusions take part in the formation of proteid yolk either directly or indirectly

In this animal no direct metamorphosis of the extrusions into albuminous yolk has been observed.

Yolk Bodies

During the last few years, opinion seems to be crystallizing on the fact that there are two types of yolk bodies, (i) Fatty yolk, and (ii) Albuminous or Proteid yolk. The origin of these yolk bodies has been a subject of much controversy among recent workers in Cytology and the views upheld by various authors are sometimes contradictory. Some ascribe the origin of fatty yolk to *de novo* formation in the cytoplasm There are a few who ascribe the formation of fatty yolk to the metamorphosis of mitochondria but most of the modern cytologists agree that fatty yolk arises directly or indirectly in relation to Golgi elements. Nath (53), Gatenby (28, 29), Ludford (45), Bhattacharya (3), Das (15), and several others uphold this view.

Gatenby and Woodger (28), Ludford (45), and Bromhall (10), showed that in Helix, Limnaea, and Patella, the fatty yolk is formed directly by the Golgi elements. Hirschler has similarly shown that in Ascidians (Ciona), the Golgi elements are directly metamorphosed into fatty yolk. The senior author (3, 4, 5) and his collaborators, have in a number of vertebrates, proved the direct or indirect transformation of Golgi elements into fatty yolk

Nath, in a series of papers (53), has strongly emphasised the fact that Golgi bodies give rise to fatty yolk In certain cases, the non-fatty chromophobic area or the vacuolar area of the Golgi vesicles is directly transformed into vacuolar fatty yolk bodies in the course of development of the oocytes (Spider, Scolopendra, Cockroach, etc); in others, they are from the very beginning fatty in nature (Luciola, Dysdercus), and grow in size to form big yolk bodies This fatty yolk is dissolved out when treated with turpentine leaving osmiophilic rims and crescents behind

Gatenby (22) and Ludford (45), in Saccocirrus and Patella respectively, have shown that the fatty yolk arises by the swelling up of the Golgi bodies

Recently Hibbard (38), and Harvey (36), have claimed that fat arises independently in the cytoplasm without any relation to Golgi bodies and mitochondria in the eggs of Discoglossus and Carcinus respectively Harvey in Carcinus observed that there was no relationship between the Golgi bodies and the fatty yolk In the animal examined by us, the fatty yolk has been observed to be formed directly by the Golgi elements

In Da-Fano preparations, the fatty yolk is early represented either by an archoplasmic area to which a dictyosome is attached or a vacuolar area surrounded by a chromophilic rim In the Champy technique, fatty yolk bodies appear as solid dull black bodies The black bodies after treatment with turpentine are readily differentiated leaving clear vacuoles with an osmiophilic rim or a crescent Intermediate stages between the fatty yolk bodies and the growing Golgi elements have also been observed Thus it is concluded, that the fatty yolk bodies are formed directly by the swelling up of the Golgi elements Most probably, as Nath conjectured, Harvey has been dealing with fat droplets and not fatty yolk bodies

The Albuminous yolk has been observed even in very young oocytes They do not seem to possess any relationship with the Golgi bodies and mitochondria and probably arise *de novo* in the cytoplasm

Parat and Hibbard have demonstrated in several animals (Perca, Discoglossus, Aplysia, etc), the relation between proteid yolk-formation and Golgi bodies Similarly, Weiner in Lithobius and Tegenaria has shown that proteid yolk is formed on the periphery of the egg, among and in intimate relation to Golgi bodies There are others, *e.g.*, King in Oniscus, Gatenby and Woodger in Apantales,

who attribute the formation of proteid yolk in relation to mitochondria

In quite a large number of animals (Invertebrates) the work carried on in this line has shown that proteid yolk is formed mostly in relation to nucleolar extrusions Nath in a series of animals (Lucicola Lithobius, Spider, Cockroach, Scorpion, Dysdercus, etc) has observed remarkable nucleolar extrusions given out by the nucleolus, which pass out to the cytoplasm and are either directly or indirectly transformed into proteid yolk.

Harvey has observed proteid yolk-formation in relation to Golgi bodies and further says, "probably it is deposited in the chromophobic part thereof." The present authors have been unable to find any existing relationships whatsoever, between the formation of proteid yolk and the Golgi elements or mitochondria in the animal under discussion

Moreover, in spite of the fact that nucleolar extrusions are present in the cytoplasm, they have never been noticed to give rise to proteid yolk bodies directly. Thus it is assumed that the proteid yolk spheres are formed *de novo* in the cytoplasm.

The centrifuge experiments also confirm the above conclusions as we notice that neither the mitochondria nor the Golgi elements have any direct relationship with proteid yolk-formation whereas the Golgi elements are in close association with the fatty yolk bodies. Thus, the conclusion is forced on us that fatty yolk is formed directly by Golgi elements while the albuminous yolk is produced *de novo* in the cytoplasm

Vital Colouration Experiments

Since the vital staining methods offer satisfactory results in this animal, it is worthwhile discussing in this paper, briefly, the supposed homology of the Golgi bodies and vacuome

Parat (65, 67, 69) with his collaborators, for the first time demonstrated the occurrence of vacuome in the animal cells by vital colouration methods and believed that the Golgi apparatus and the vacuome were homologous structures Further, the examination of salivary glands, pancreas, etc, led Parat to the conclusion that the Golgi apparatus is constituted of a system of vacuoles (Vacuome) in which "Granules de secretion" are produced by a process of condensation. He observed that the Golgi bodies are really the vacuoles which are stained with dilute neutral red, and that the osmiophilic rim or crescent is an artefact or is constituted of some special kind of chondriosome, lipoidal in nature, which is associated with the vacuole occasionally To these special chondriosomes he gives the name of "Lepidosomes' Thus, according to this view, the vacuolar space represents the vacuome ("Golgi body"), which may be surrounded by special chondriosomes called Lepidosomes

Recently, Bowen (9) in plant cells, and Gatenby (25) in male gorm cells of animals, have vehemently criticised Parat's Lepidosome theory Gatenby, taking into consideration the definition of the Golgi apparatus maintains that "It is an argentophil structure discovered in the nerve cells as such by Golgi" (26) He further adds that the so-called Parat's "*Lepidosome*" is the real *Golgi element* associated with an archoplasmic area or the vacuole In the male germ cells some of the vacuoles are secreted by the Golgi elements and they collectively form congeries of vacuoles—the "Vacuome," staining with the dilute neutral red solution Thus he contradicted the view held by Parat that Golgi bodies are vacuoles whereas the argentophil structure is an artefact

Very recently, Beams and Goldsmith (1), in the salivary glands of Chironomus larva, observed that probably the "neutral red bodies are in reality the secretory inclusions which have been coloured by the dye." They conclude that the neutral red bodies cannot represent the Golgi

bodies, the latter being argentophil in structure and are never found to be coloured with neutral red. Bhattacharya and Das in the ovary of the young pigeons (6), have found that Vacuome is quite a different structure and cannot be confused with the discrete Golgi elements as both these structures can be seen at the same time in vital examination lying separately as distinct structures. There appears to be, however, a close relationship between this "Vacuome" and the Golgi crescents, the latter being sometimes associated with the former.

Nothing definite can be said yet as to the behaviour and function of these neutral red staining bodies

A TABULAR REPRESENTATION OF THE VARIOUS CYTOPLASMIC INCLUSIONS AND THEIR RELATIONS IN REGARD TO VITELLOGENESIS IN SCYLLA SERRATA.

Oogonial stages	Early oocytes	Fully developed oocytes
Golgi Bodies—		
Form —Granular	(1) Vesicular - chromophilic rim and chromophobic centre (2) Crescent-shaped (dictyosomes)	Same as in early oocytes
Disposition —A few in number, adjacent to the nucleus	Formation of the "Yolk nucleus of Balbiani"	Scattered in the cytoplasm
Function —Nil	Swollen up Golgi elements with one or two fatty yolk bodies	Many fatty yolk spheres
Mitochondria—		
Form —Granular and dusty	Granular and dusty	Granular and dusty
Disposition —Few, adjacent to the nucleus	Formation of "Yolk nucleus of Balbiani"	Scattered throughout.
Function —Apparently nil	Apparently nil	Apparently nil

Oogonial stages	Early oocytes	Fully developed oocytes
Nucleolus—		
Form —round small bodies	Large, oval.	Large, oval
Disposition —Many nucleoli embedded in the nucleolymph, tendency to arrange themselves around the nuclear membrane	One becomes prominent Others plaster themselves round the periphery of the nucleus	Basophil nucleolus gives out nucleolar extrusions which become oxyphil in the ooplasm
Function —Apparently nil, but may influence the indirect synthesis of the proteid Yolk		
Fatty Yolk —None	Few	Numerous
Alb. Yolk None	A fair number	Numerous

A TABULAR REPRESENTATION OF YOLK FORMATION IN ANIMALS

Genus or animal	Author	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present.
Grantia	Gatenby, J B.		In ground protoplasm	
Ascaris ...	Hirschler.	Cytoplasm	Mitochondria	..
Saccocirrus	Gatenby ...	Golgi bodies	Nucleolar extrusions.	Yes.
Peripatus	King	Formed in groups but its source has not been determined.	...	Probable

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Form —Granular and dusty	Granular and dusty	Granular and dusty
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Function —Apparently nil	Apparently nil.	Apparently nil.

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Peripatus .	Kiog ..	Formed in groups but its source has not been determined	...	Probable

Genus or animal	Author	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present.
Circinus	Harvey	Cytoplasm (independently)	Golgi bodies.	Probable
Oniscus	King	Golgi bodies	Mitochondria	...
Limulus	Gardiner	Nucleolar extrusions	Interaction of Golgi bodies, mitochondria, and nucleolar extrusions	Present
Palamnaeus	Nath	Golgi bodies	None	None
Scorpion	Nath	Do	Nucleolar extrusions	Yes
Cockroach	Nath and Piere Mohan	Do	Do	Do.
Scolopendra	Nath and Hussain.	Do	Do.	Do
Luciola ..	Nath and Mehta.	Do	Do	Do
Lithobius.	King	Probabaly Golgi bodies	Do.	Do
,,	Nath	Apparently Golgi bodies	Do	Do
,,	Weiner	Cytoplasm ..	Indirectly from Golgi bodies.	
Tegenaria	Weiner	Vitelline layer	Golgi bodies	...
Spider (Crossopriza)	Nath	Golgi bodies	*De novo* in the cytoplasm.	None.

Genus or animal	Author	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present
Culex ..	Nath .	Fat deposits in microspheres.	Microspheres or proteid yolk bodies.	..
Apantales	Gatenby	...	Mitochondria and secondary nuclei	...
Tenthredinidæ	Gresson .	Golgi bodies	Nucleolar extrusions.	Yes
Daphnia ..	Hill and Gatenby	Golgi bodies	None	None.
Nepa	Steopoe ...		Golgi bodies	...
Dysdercus	Nath ..	Golgi bodies	Nucleolar extrusions.	Yes
Helix	Gatenby .	Golgi bodies	Probable	None.
Patella ...	Ludford	Golgi bodies	...	Yes.
Pila globosa	Bhattacharya and Lal	Golgi bodies	Nucleolar extrusions.	Yes
Ostrea ..	Rai H. R. Singh	Golgi bodies	Absent ...	None.
Calanus	Hilton, I. F.	None	Mitochondria	Yes.
Ophiocephalus	Narain, D.	Golgi bodies	Mitochondria	Yes.
Diaceglossus.	Hibbard, H.	*De novo* in the cytoplasm.	Golgi bodies	...
Rana .	Narain, D.	Golgi bodies	Mitochondria	Yes.
Tortoises .	Bhattacharya.	Golgi bodies	Mitochondria	Yes.

Genus or animal	Author	Fatty yolk	Albuminous yolk	Nucleolar extrusions, if present
Fowl	Brambell	Possibly under the influence of Golgi bodies	Mitochondria	None.
Birds (Pigeon)	Das, R. S	Golgi bodies	Mitochondria	None
Lepus	Del Rio Hortega	Mitochondria and Cytoplasm		
Lemur	Rao, S	Nucleolar emissions	Mitochondria and Golgi bodies	Yes.

SUMMARY

(1) The Golgi apparatus in Scylla Serrata is revealed best by Da-Fano's Cobalt nitrate method and Ludford's technique

(2) The Golgi apparatus consists of discrete crescent-shaped or spherical bodies as revealed in fixed preparations as well as by Intra-vitam examinations The spherical Golgi body may be differentiated into a chromophilic rim and a chromophobic centre while the dictyosome appears as an osmiophilic crescent attached to an archoplasmic area

(3) In the oogonial stages the Golgi elements appear in the form of a few black granules lying in the clear and homogeneous cytoplasm adjacent to the nucleus

(4) In the early oocytes the Golgi elements form a juxta-nuclear complex mass—the "Yolk-nucleus of Balbiani" which has been regarded as the focus of growth and dispersal of Golgi bodies.

(5) Gradually, the Golgi elements begin to get detached from the compact mass and disperse in the cytoplasm till in a full-grown oocyte these bodies are seen scattered throughout the cytoplasm

(6) During the formation of Yolk-nucleus some Golgi elements swell up to form fatty yolk bodies by deposition of fat inside the chromophobic or archoplasmic area

(7) Fatty yolk bodies appear as solid black bodies in Osmic preparations and are readily dissolved when treated with turpentine leaving clear vacuoles with an osmiophilic rim or a crescent.

(8) Mitochondria are revealed best by Regaud, Regaud-Tupa and Champy-Nassonov techniques.

(9) Like the Golgi elements they also occur in the "Yolk-nucleus" area, whence they migrate round the nucleus till gradually they disperse throughout the cytoplasm. They are always granular and dusty and have never been noticed to swell up. No filamenter forms have been observed

(10) Nucleolar phenomenon is best exhibited in Bouin preparations stained with Mann's methyl-blue-eosin.

(11) From the very beginning the nucleoli have a tendency to plaster themselves to the nuclear membrane leaving behind

a prominent basophil nucleolus The former pass out into the cytoplasm as such or fragment into nucleolar extrusions

(12) The basophil nucleolus gives out basophil bodies which pass out from the nucleus into the cytoplasm. During this period they get transformed into oxyphil nucleolar extrusions which may indirectly influence the synthesis of proteid yolk.

(13) Occasionally, the whole of the nucleolus is seen to come out of the nuclear membrane into the ooplasm without apparently rupturing the nuclear wall The significance of this is unknown but probably it buds off nucleolar extrusions in the ooplasm.

(14) The true yolk or proteid yolk is formed "de novo" in the cytoplasm Mitochondria play absolutely no part in vitellogenesis

(15) In the Intravitam examinations, patches of neutral red staining vacuoles have been observed which lie quite distinct and separate from the Golgi bodies These are the patches of Parat's " Vacuome " Their behaviour and functions are yet to be discovered

EXPLANATION OF PLATES

The drawings were made under Leitz Abbe Camera Lucida

Figs 1-6 Da-Fano preparations stained in Safranin and Light green Sections were cut 6 μ in thickness

Fig 1. The young oogonial stage showing a few discrete Golgi granules

Figs 2 and 3 Early oocytes in which the Golgi granules have a tendency to lie on one side of the nucleus

Fig. 4 An oocyte showing the " Yolk-nucleus of Balbiani " formed by the Golgi bodies.

Fig 5 An oocyte in which the Golgi bodies have begun to detach themselves from the Yolk-nucleus of Balbiani and scatter in the cytoplasm

Fig 6 Showing a well advanced oocyte in which the Golgi elements have scattered throughout the cytoplasm in the form of vesicles and crescents Fatty yolk bodies are also visible formed by the Golgi bodies

Figs 7-14 Ludford preparations stained in Champy-Kull

Fig 7 represents an early oogonium showing two or three Golgi granules in the clear homogeneous cytoplasm.

Figs 8-10 represent early oocytes in which the Golgi granules increase in number and lie juxta-nuclear in position.

Fig 11 An oocyte showing the formation of the "Yolk-nucleus of Balbiani"

Fig 12. An oocyte where the Golgi bodies give rise to some fatty yolk bodies The yolk-nucleus has begun to disseminate in the cytoplasm

Fig 13 Showing the detached Golgi vesicles and dictyosomes scattered throughout the cytoplasm around the nucleus.

Fig 14 Showing a fully developed oocyte in which the Golgi elements have dispersed throughout the cytoplasm Besides the fatty yolk bodies, some albuminous yolk bodies are also seen, stained cherry red in acid fuchsin

Figs 15-23 Champy-Nassonov preparations stained in Champy-Kull. Sections 5 μ in thickness.

Fig. 15. An advanced oogonium showing two or three dusty granules of mitochondria

Figs 16 and 17 represent early oocytes where the mitochondrial granules increase in number and lie adjacent to the nucleus.

Fig 18 An oocyte where the mitochondria occur in the "Yolk-nucleus of Balbiani" Some albuminous yolk bodies are also visible stained in acid fuchsin.

Figs. 19 and 20 represent oocytes where the mitochondrial granules detach from the yolk nucleus and form a peri-nuclear zone

Figs 21, 22 and 23 represent the well advanced oocytes where the mitochondria increase in number and scatter throughout the cytoplasm Albuminous yolk bodies are fairly big in size and dispersed in the cytoplasmic area

Figs 24-27. Regaud preparations stained in Champy-Kull Sections 5 μ in thickness

Fig 24 An early oocyte showing some mitochondrial granules lying adjacent to the nucleus.

Fig. 25 Showing the formation of the "Yolk-nucleus of Balbiani."

Figs 26 and 27. Showing the dispersal of the mitochondria in the cytoplasm as in Champy-Nassonov preparations.

Figs 28-31. Bouin preparations stained in Mann's methyl-blue-eosin Sections 5 μ in thickness.

Fig 28 Showing an oocyte in which there are many nucleoli within the nucleus, and one of them being prominent

Fig 29 Showing an oocyte where the nucleoli plaster themselves round the nuclear membrane leaving a prominent basophil nucleolus stained deep red in Mann's methyl-blue-eosin

Fig 30 Showing some oxyphilic nucleolar extrusions in the cytoplasm coming through the nuclear membrane Oxyphil bodies are stained lighter in the stain.

Fig 31 Showing a well advanced oocyte in which the cytoplasm is fully packed with the nucleolar extrusions

Figs 32-34 represent the Fatty yolk formation Di-Fano preparations stained in Safranin-Light green

Fig 32 Showing the vacuolar fatty yolk spheres each with an associated Golgi crescent or vesicle, in the region of the yolk-nucleus

Figs 33 and 34 Showing the oocytes where the intermediate stages between the Golgi elements and the fatty yolk bodies are seen (swollen up Golgi bodies)

Fig 35 shows a Champy-Nassonov preparation stained in Champy-Kull Representing the albuminous yolk bodies stained cherry red in acid fuchsin

Fig 36 represents the centrifuged material fixed in Champy-Nassonov followed by Champy-Kull Three separate zones of mitochondria, albuminous yolk, and Golgi bodies with associated fatty yolk bodies are visible individually

Figs. 37 and 38 represent oocytes in "Bouin" stained with Mann's methyl-blue-eosin, showing the occasional shifting of the nucleolus into the cytoplasm apparently without rupturing the nuclear wall In the cytoplasm it presents an oxyphilic consistency

Figs 39, 40, 41 represent oocytes as seen in the Intra-vitam examination with Neutral red These represent the structure and dispersal of the 'Vacuome' patches, formed of congeries of small vacuoles stained red in neutral red

Figs 42 and 43 represent oocytes studied afresh in 2 per cent osmic acid. These show the fatty yolk bodies and the Golgi crescents and vesicles with an osmiophilic rim and a clear chromophobic area.

LETTERING

Gb	Golgi bodies.
Gb_1	Swollen up Golgi bodies
F.	Fat
F y	Fatty yolk.
M.	Mitochondria
A y.	Albuminous yolk.
N.	Nucleus
Nu	Nucleolus
N E.	Nucleolar extrusions.
Y Nuc	Yolk-nucleus of Balbiani
B Nu	Basophil nucleolus
O Nu	Oxyphil nucleolus
A Nu.	Amphophil nucleolus
Vo.	Vacuome

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Plate No 1

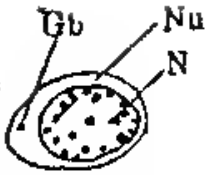


Fig 1.

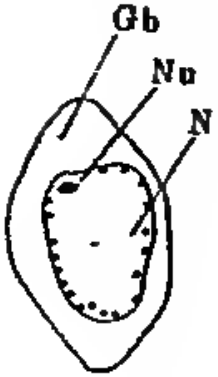


Fig 2

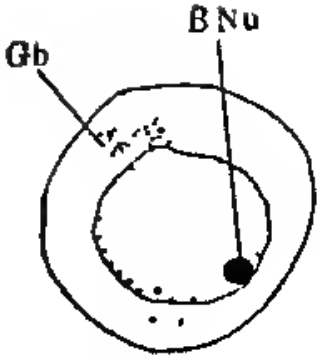


Fig 3

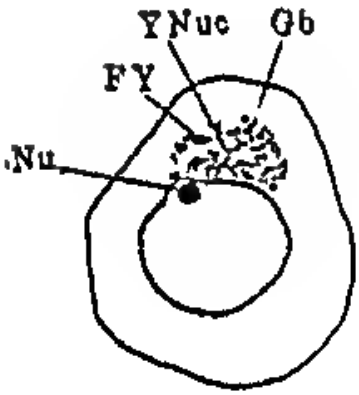
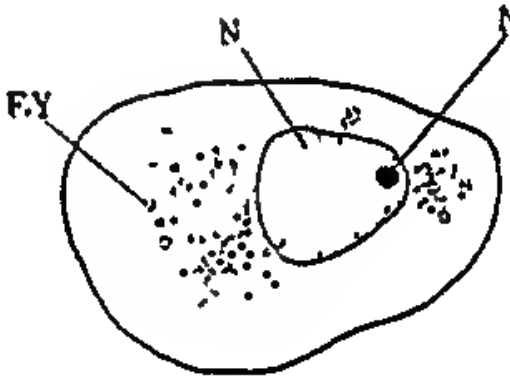


Fig.4.



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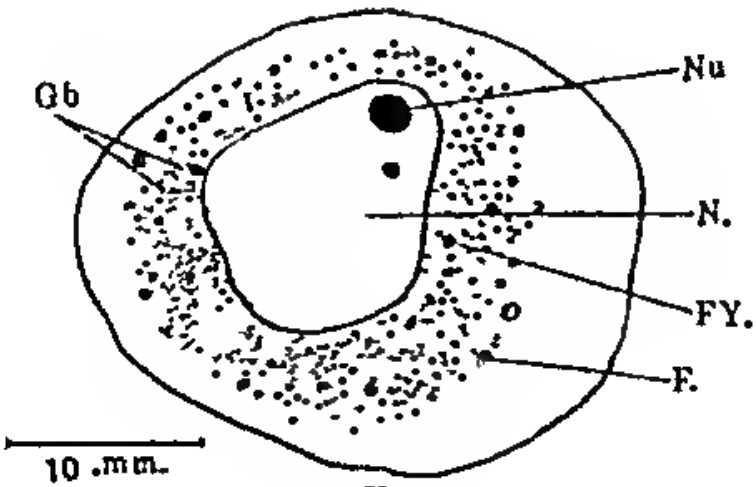


Fig 6
Da-Fano

Plate No 1

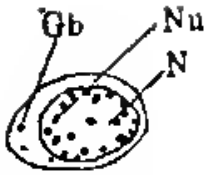
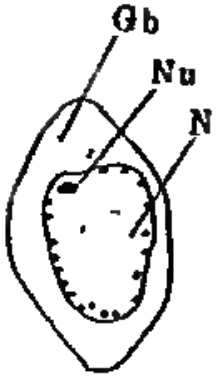


Fig 1.



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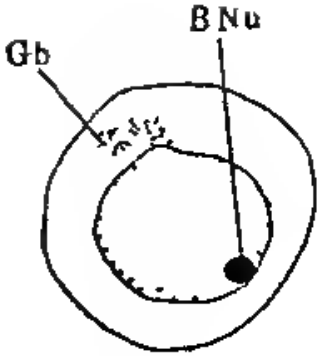
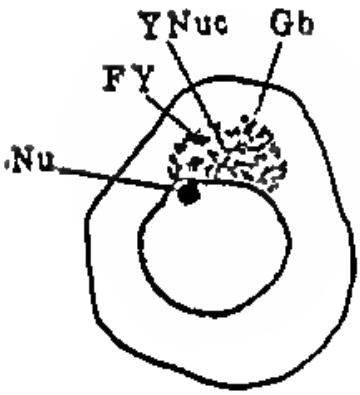


Fig 3



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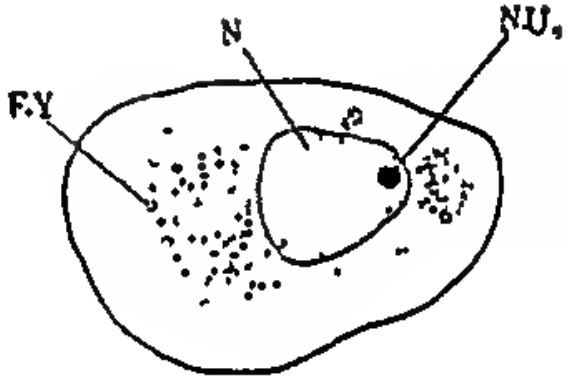

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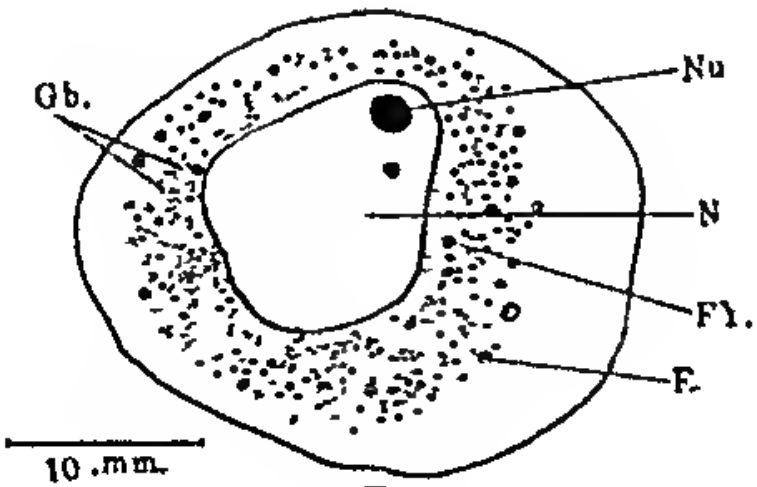
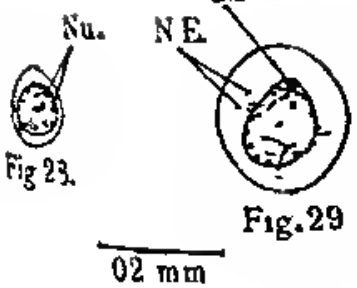
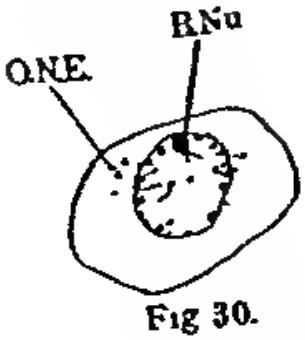
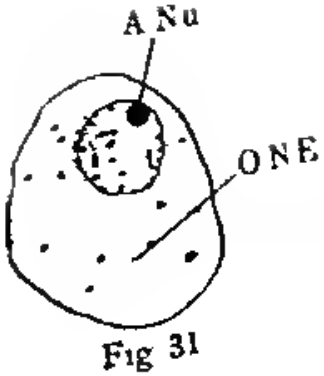


Fig 6
Da Fano.

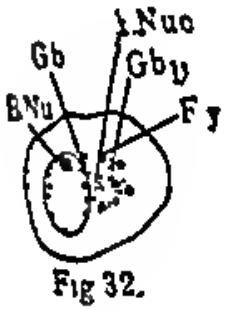
Plate No. 4

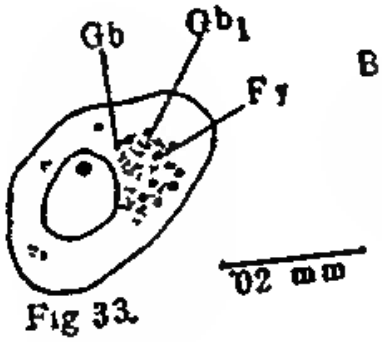


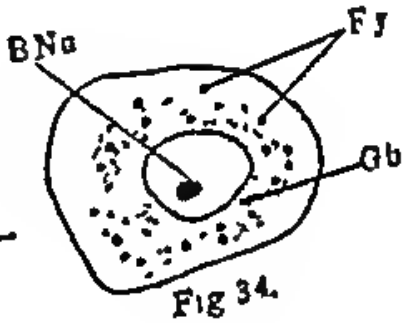




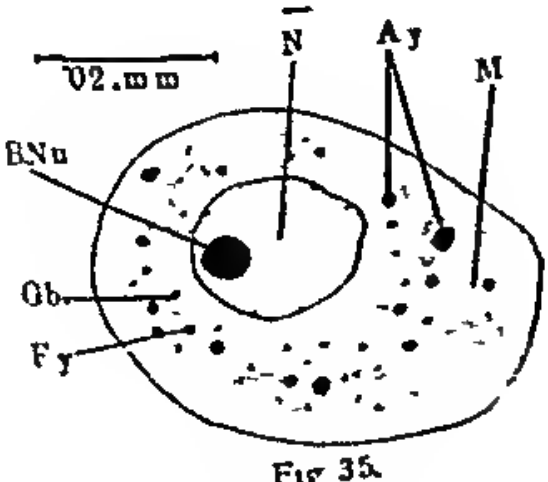
Bouin-Mann's Methyl-Blue Eosin.





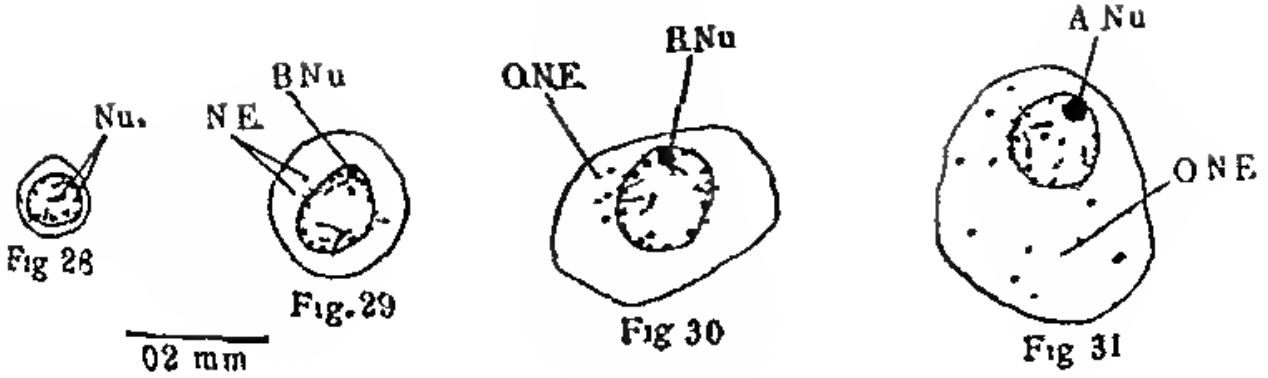


Da-Fano

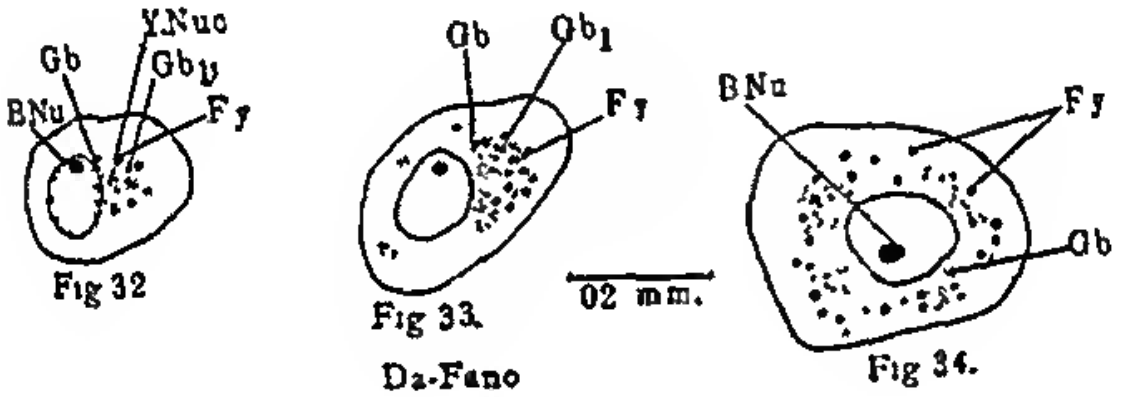


Champy-Nassonov-Champy-Kull.

Plate No. 4



Bouin Mann's Methyl-Blue Eosin



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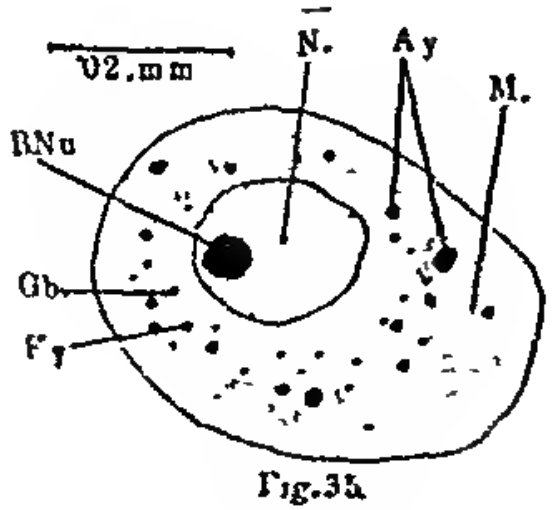

Champy-Nassonov-Champy-Kull.

Plate No 5

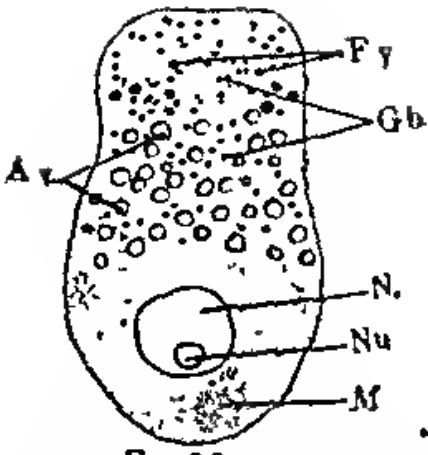

Fig 36

Centrifuge-Champy-Nassonov
Champy Kull

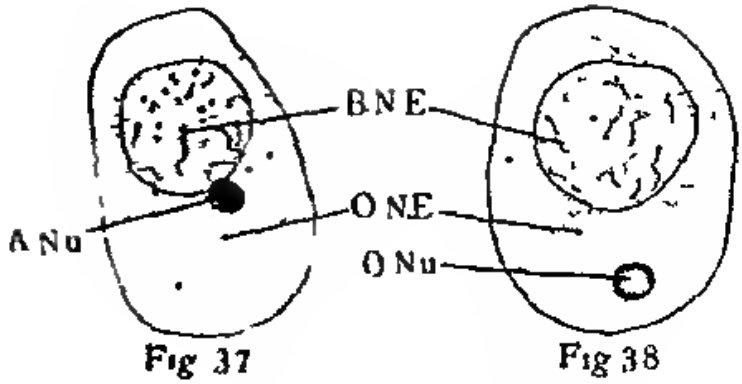

Fig 37 Fig 38

02 mm

Bouin Mann's Methyl-Blue Eosin

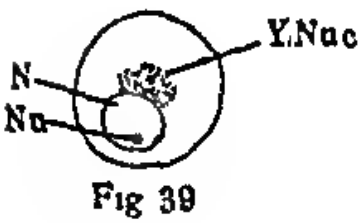

Fig 39

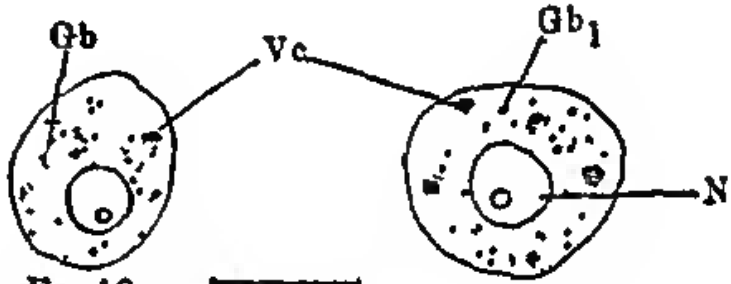

Fig 40 02.mm. Fig 41

Intra Vitam-Neutral Red

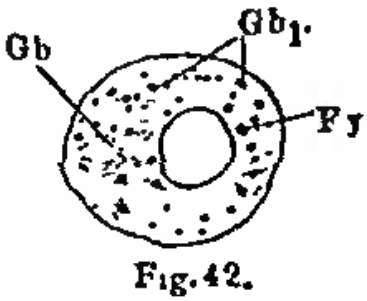

Fig.42.

02.mm.

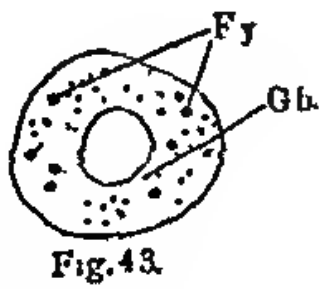

Fig.43.

Intra-Vitam-2% Osmic-Acid.

SECTION II

CHEMISTRY

IODINE VALUE OF SATURATED FATTY ACIDS AND THEIR SALTS

BY

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Research Scholar, Chemistry Department

In 1898 Wijs[1] proposed a method for the determination of Iodine Value in which iodine trichloride was made to be absorbed by oils in presence of glacial acetic acid. Hubl, Winkler and others[2] have proposed alternative methods for the determination of iodine numbers and since then, various investigators have determined the iodine number for almost all vegetable and animal fats, and unsaturated fatty acids Up till now it had been supposed that unsaturation is an essential condition for the absorption of iodine and this was the reason why only the unsaturated fats and oils have been investigated

In a previous communication from this laboratory, Palit and Dhar[3] studied the oxidation of the salts of saturated fatty acids by passing a current of air through their solutions in presence of various inductors in tropical sunlight. They found that the amount of the fat that oxidised can easily be estimated by determining the amount of absorption of iodine trichloride by fat before and after the experiments. From these results we are led to think that

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[1] Wijs Ber, *31*, 750 (1898), Ztschr Nahr. U Genus, *4*, 913 (1901).

[2] Rowland Williams J. Soc. Chem. Ind, *19*, 300 (1900), Tolman and Munson J. Amer. Chem Soc., *25*, 244 (1903); Aschman. Chem. Ztg, *22*, 69, 71 (1898); Margosches, Barn and Wolf. Z Angl Chem, *62*, 178 (1923). Weiser and Donath: Zeitsch Nahr Genussm, *28*, 65 (1914).

[3] Palit and Dhar. J Phys Chem, *34*, 711 (1930).

saturated fatty acids do also possess a specific iodine number, howsoever small it may be, in comparison to that of the unsaturated compounds

In the present paper, I have thoroughly investigated this problem and have determined the iodine value of the following substances :—

Propionic Acid.
Sodium Propionate
Butyric Acid.
Sodium Butyrate
Stearic Acid
Potassium Stearate.
Palmitic Acid

EXPERIMENTAL

In the determination of the iodine value the procedure recommended by Wijs was followed. The following reagents were prepared :—

1. Iodine Solution—This was prepared by dissolving thirteen grams of iodine in one litre of glacial acetic acid Pure glacial acetic acid, which did not give a green colour on heating with potassium dichromate and sulphuric acid after prolonged standing (for about six to eight hours), was used 10 c.c of this solution were titrated with a standard solution of sodium thiosulphate. Chlorine, purified and dried by washing through concentrated sulphuric acid was led into the iodine solution, till it changed colour and its iodine content was doubled The solution was kept for 24 hours before being used (Lewkowitch)

2 Standard sodium thiosulphate solution—It was prepared by dissolving a carefully weighed amount of the

pure salt in distilled water and making it up to a known volume. A fairly dilute solution of about 0·025N strength was employed

3 A 10·0 per cent potassium iodide solution was used.

4 A fresh starch solution of about 1·0 per cent strength was prepared for use as an indicator, by pouring an emulsion of starch in cold water in nearly boiling water contained in a beaker, and stirring it

A carefully weighed quantity of the pure fatty acid or one of its salts was dissolved in some pure glacial acetic acid, and the solution was then made up to 100 c.c. by adding the iodine solution as prepared above Immediately after preparation, 10 c.c. of this solution was taken in a glass-stoppered Jena bottle, 10 c.c. of a 10·0 per cent potassium iodide solution and 25 c.c. water added, and the whole titrated with a standard solution of potassium thiosulphate This gave the blank reading 10 c.c. of the solution of the fat were taken in each of a number of glass-stoppered Jena bottles and kept in an electrically-driven rocking machine for a number of hours in order to keep the solution in a constant state of agitation throughout. The bottles were covered with a thick dark-coloured paper to protect them from diffused light They were taken out as required, 10 c.c. of a 10 per cent potassium iodide solution and 25 c.c. water added, and the whole titrated with a standard sodium thiosulphate solution From this data, the iodine value was calculated in the usual manner.

Table I

Absorption of Iodine by Propionic Acid

8 1228 gms of propionic acid was dissolved in glacial acetic acid, and the solution was then made up to 100 c c. with the iodine solution as prepared above.

Concentration of the sodium thiosulphate solution used = N/38 29

10 c c of the above solution required 21·0 c.c sodium thiosulphate (Blank)

10 c c of the above solution were titrated with hypo after shaking mechanically for .—

Hours	N/38 20 hypo required	Iodine Value
15	20 4 c c	0 25
24	20·3 c c	0·29
41	t7 7 c c	t 35
47	t7 25 c c	t 53

Before each titration, 10 c c. of a 10 per cent potassium iodide solution and 25 c.c water were added to each bottle. The starch solution was added near the end of the titration when the colour of the solution became very pale

Table II

Absorption of Iodine by Sodium Propionate

1 5518 gms of sodium propionate was dissolved in glacial acetic acid, and the solution was made up to 100 c c. by adding iodine solution.

Concentration of the hypo-solution used = N/38·29.

10 c c. of the above solution were taken in a glass-stoppered bottle, 10 c c of n 10 per cent KI solution and 25 c c. water were added to it. It required 12 3 c.c. of N/38 29 $Na_2S_2O_3$ (Blank)

10 c c of the fat solution were taken in each of the two glass-stoppered bottles and were shaken mechanically

for a number of hours. After that 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$

Hours	N/38·29 $Na_2S_2O_3$ required	Iodine Value
6	7·8 c.c	0·96
22	7·2 c.c.	1·1

Table III

Absorption of Iodine by Butyric Acid

1·6369 gms. of butyric acid were dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution

Concentration of $Na_2S_2O_3$ solution used = N/38·29

10 c.c. of the solution of the fat were taken in a glass-stoppered bottle, 10 c.c. of a 10 per cent KI solution and 25 c.c. water were added to it. It required 27·5 c.c. of N/38·29 $Na_2S_2O_3$ (Blank).

10 c.c. of the fat solution were taken in each of the three glass-stoppered Jena bottles, and were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI and 25 c.c. water were added to each bottle, which were then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ required	Iodine Value
24	27·25 c.c.	0·43
47	27·10 c.c.	0·69
72	26·90 c.c.	1·13

Table IV

Absorption of Iodine by Sodium Butyrate

1·5767 gms. of sodium butyrate were dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution.

Strength of sodium thiosulphate solution = N/38·29.

10 c.c. of the above solution were taken in a glass-stoppered bottle, 10 c c of 10 per cent KI solution and 25 c.c. water were added to it. It required 11·4 c.c. of N/38·29 $Na_2S_2O_3$ (Blank)

10 c.c. of the solution of the fat were taken in each of the four glass-stoppered bottles, which were shaken mechanically for a number of hours After that, 10 c c of 10 per cent KI solution and 25 c.c water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ used	Iodine Value
6	8·3 c c.	0·65
23	8·2 c.c.	0·67
29	8·2 c.c	0·67
45	8·0 c c	0·72

Table V

Absorption of Iodine by Stearic Acid

1·2357 gms. of stearic acid was dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution

Concentration of $Na_2S_2O_3$ solution used = N/38·29

10 c.c. of the fat solution were taken in a glass-stoppered bottle, 10 c.c. of 10 per cent KI solution and 25 c c. water were added to it It required 27·3 c c. of N/38·29 $Na_2S_2O_3$ (Blank)

10 c.c. of the solution of the fat were taken in each of the three glass-stoppered bottles, which were shaken mechanically for a number of hours After that, 10 c.c of 10 per cent KI solution and 25 c c. water were added to each bottle, and then titrated with $Na_2S_2O_3$.

Hours	N/38·29 $Na_2S_2O_3$ used	Iodine Value
16	26·9 c.c.	1·4
25	26·8 c c	1·7
39	26·6 c.c	2·4

Table VI

Absorption of Iodine by Palmitic Acid

1·0983 gms of palmitic acid was dissolved in glacial acetic acid, and the solution was made up to 100 c.c. by adding iodine solution

Strength of $Na_2S_2O_3$ solution = N/38 29

10 c c. of the fat solution was taken in a glass-stoppered bottle, 10 c.c. of 10 per cent KI solution and 25 c.c water were added to it. It required 42 9 c.c. of N/38 29$Na_2S_2O_3$ (Blank).

10 c c. of the solution of the fat were taken in each of the four glass-stoppered bottles, which were shaken mechanically for a number of hours. After that, 10 c.c. of 10 per cent KI solution and 25 c.c. water were added to each bottle, and then titrated with $Na_2S_2O_3$

Hours	N/38 29 $Na_2S_2O_3$ used	Iodine Value
15	41·9 c c.	0 30
24	41·7 c c.	0·36
38	41·3 c c	0 48
50	40·8 c c.	0·63

Table VII

Absorption of Iodine by Potassium Stearate

0 5642 gms of potassium stearate was dissolved in glacial acetic acid, and the solution was made up to 100 c c. by adding iodine solution.

Concentration of $Na_2S_2O_3$ solution used = N/44 9

10 c c of the fat solution was taken in a glass-stoppered bottle, 10 c c. of 10 per cent KI solution and 25 c c. water were added to it. It required 27·2 c.c of N/44·9$Na_2S_2O_3$ (Blank)

10 c.c. of the solution of the fat were taken in each of the three glass-stoppered bottles, which were shaken mechanically for a number of hours After that 10 c c of 10 per cent KI solution and 25 c c. water were added to each bottle, and then titrated with $Na_2S_2O_3$

Hours	N/44·9 $Na_2S_2O_3$ used	Iodine Value
31	25·5 c c	0·85
49	24·7 c c	1·25
73	21·1 c c	1·55

DISCUSSION

From the results recorded in the previous tables, it would be seen that sufficient quantity of iodine is absorbed by such saturated compounds as butyric acid, propionic acid, stearic acid, and palmitic acids and also by their sodium and potassium salts. The experiments have been repeated many times and the possibility of the experimental error has been avoided as far as possible It can be said with confidence that saturated acids also possess an iodine number similar to that in the case of unsaturated acids It is also clear from my observations that the iodine value of these substances continuously increases with time. It is only after the lapse of a considerable time, that the iodine value, in the case of saturated compounds, attains a maximum. In the case of unsaturated compound, it has been generally observed that the maximum condition is reached within a few hours.

Margosches, Friedmann, Tschörner* observed something like the iodine super-value in the case of olive, castor and linseed oils and oleic, ricinoleic, and linoleic acids in aqueous alcoholic solutions. It shows that unsaturated acids are,

* Margosches, Friedmann and Tschörner Ber. *58* (B), 794 (1925)

in the first instance, converted into saturated iodine compounds which are capable of further oxidation. I am of opinion that the iodine number for the saturated acids can also be explained in a similar way. The mechanism proposed by the above authors for the super-iodine value is as follows :—

(1) $I_2 + H_2O = HI + HIO$.

(2) $R'CH{:}\,CH.CH_2R'' + HIO = R'CHI.CH(OH).CH_2R''$.

(3) $R'CHI.CH(OH).CH_2R'' + HIO = R'CHI.CH(OH).CH(OH)R'' + HI$.

In the first step iodine is actually added to the unsaturated molecule, while in the second step the iodine acts as an oxidising agent. Similarly, the iodine absorption of saturated fatty acids can be explained in the following way :—

(1) $H_2O + I_2 = HI + HIO$.

(2) $RCH_2CH_2COOH + HIO = RCH_2CH(OH).COOH + HI$.

The above authors also observed that for the super-iodine value the substances must remain in contact for twenty-four hours or a longer period. The iodine numbers which I have obtained for saturated compounds do not exceed 2·5 in value, while in the case of unsaturated substances they are often as high as 200. This very fact shows that the iodine number of saturated compounds is not primary, but secondary.

The iodine value has been, up till now, associated with unsaturation and it has been regarded as a reliable method for the estimation of unsaturation. It may be stated, however, that the iodine value, thus determined, gives the combined values due to unsaturation and the secondary oxidation of saturated compounds.

The results recorded in the previous tables also show that there exists no relationship between the iodine value

and the number of carbon atoms of the saturated fatty compound Acetic acid does not appear to absorb iodine to any marked extent even when kept in contact for 45 hours as is seen from the following results :—

Table VIII

Four bottles were taken each containing 5 c c. iodine solution as prepared above. They were titrated with N/38·29 hypo after a number of hours.

No of hours	N/38·29 hypo used
Immediately	22·55 c.c
After 20 hrs	22·6 c.c.
After 26 hrs	22·6 c c
After 45 hrs	22·6 c c.

However, as soon as the number of carbon atoms increases, the power of absorbing iodine is at once exhibited It is strange that propionic and stearic acids possess a remarkably higher value than the butyric and palmitic acids In the case of propionic and butyric acids, the sodium salts possess higher value than the corresponding free acids From this it appears that sodium salts are more liable to oxidation.

SUMMARY

1 The iodine values for the following saturated fatty acids have been determined —propionic, butyric, palmitic and stearic

2. It has been shown that the saturated fatty acids also possess an iodine value, which, however, continuously increases with time.

3 The iodine value of the sodium salts of the above acids has also been determined, and it has been shown that the salts generally possess a higher value than the corresponding acids

4 There appears to be no relationship between the iodine value and the constitution of the compound

5. A mechanism for the absorption of iodine has been suggested and it is believed that the iodine value is not due to the addition of iodine to the structure of the molecule, but to the oxidation of the methylene group

colourless transparent jelly On warming, however, the jelly was transformed to a gelatinous precipitate

Klosky and Marzano[1] prepared firm transparent jellies by neutralising slowly an acid solution of titanium dioxide with sodium, potassium or ammonium carbonates Recently, Bhatia and Ghosh[2] obtained a sol of titanic acid by dialysing a solution of titanium tetrachloride in water (*Cf.* Majumdar J Indian Chem Soc, *6*, 337 (1929) They observed that this sol sets to a jelly by the addition of electrolytes The sol prepared by them, however, appears to be positively charged

Molybdic Acid.—Not much work appears to have been done on molybdic acid jellies It has been observed that on addition of suitable concentrations of hydrochloric acid to a strong solution of ammonium molybdate, the molybdic acid is precipitated in the form of a solid opaque jelly.

The Jellies of Basic Hydroxides

The most common of the inorganic hydroxide jellies are those of iron, chromium, aluminium, tin, zirconium, copper, mercury, manganese, scandium, erbium, cerium and non-aqueous gel of nickel

Ferric Hydroxide Jelly—Grimaux[3] added an alcoholic solution of ferric ethylate to an excess of water, which on hydrolysis yielded colloidal ferric oxide. The sol coagulated spontaneously on standing for some time at the room temperature and more rapidly on heating or adding electrolytes, like potassium or barium chlorides or sulphuric acid, in some cases forming a transparent jelly, provided the sol is not agitated during the coagulation Even dilute sols gave firm jellies Contraction took place in the jellies, however, in the cold and this too, very rapidly at higher temperatures

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[1] J Phys Chem., *29*, 1123 (1925)
[2] J Indian Chem. Soc *7*, 687 (1930)
[3] Compt Rend., *95*, 105, 1434 (1884).

The ferric hydroxide sol prepared by the Graham's method does not give jellies, but if the concentration be sufficiently high, a jelly may be formed Schalek and Szegvary[1] added electrolytes in amounts below their precipitation values to the colloidal solutions containing 6 to 10 per cent ferric oxide and allowed the sols to stand quietly After a time, the mixture set to a jelly which was almost as transparent as the original sol This jelly slowly developed opalescence It also exhibited the phenomenon of thixotropy, *i.e.*, the gel on shaking was transformed to sol which on standing re-formed the gel

Grimaux[2] obtained a firm jelly by dialysis of a negative sol prepared by peptisation of hydrous oxide with alkali in the presence of glycerine If ammonia were used instead of caustic alkali, and the sol exposed to air, the slow loss of the peptising agent by evaporation, also resulted in the precipitation of a jelly. Fischer[3] prepared a firm jelly by the prolonged dialysis of a sol containing but one per cent of iron Browne obtained a jelly simply by allowing a part of the water to evaporate slowly from a concentrated Graham sol of high purity

Dhar and Chakravarti[4] observed that various metallic hydroxide jellies can be prepared by adding sodium acetate to a metallic nitrate or chloride, and allowing the hydroxide to coagulate in the presence of ammonium sulphate, and also regulating the hydrogen ion concentration by the addition of suitable amounts of ammonia.

In a recent communication, Prakash and Dhar[5] have investigated the formation of this jelly by the above method in details The jelly has been prepared by adding varying *amounts of* 3·54 *N* sodium acetate to *M*/2 ferric chloride

[1] Kolloid-Z , *32*, 318; *33*, 326 (1923).
[2] Compt. Rend , *98*, 1485 (1884)
[3] Biochem Z., *27*, 243 (1910).
[4] Z. Anorg Chem , *168*, 209 (1927).
[5] J Indian Chem. Soc , *7*, 591 (1930).

this jelly We have observed that the jelly is best prepared by adding a sufficient amount of 3·54 N sodium acetate to M/2 solution of ferric chloride in presence of small amounts of 2M ammonium sulphate The mixture is allowed to stand for about an hour and then some ammonia is added to it If the mixture is not allowed to stand for sufficient time before the addition of ammonia, it will cause an immediate precipitation and no jelly would be obtained

The clear mixture thus obtained develops opalescence and if the concentrations of the constituents are suitable, a jelly would be formed The time of setting of the jelly would depend upon the relative concentrations of the reactants, as is shown in the following table :

TABLE II

Total Volume—5 c c

M/2 $FeCl_3$	3·54N sodium acetate	2M Ammonium sulphate	5·81N Ammonia	Observation
0 c	c c	c c.	c.c	
2 0	0·7	0 5	0 7	No jelly
2 0	1·0	0 5	0 7	Translucent jelly in 2½ hrs
2 0	1·3	0 5	0 7	Translucent jelly in 1½ hrs
2 0	1·5	0 5	0 7	Clear solution, no jelly
2 0	1 5	0 5	1 0	Firm opaque jelly in 2 hrs.
2 0	1 3	0 2	0 7	Translucent jelly in 1 hr.
2 0	1 3	0 7	0 7	Opaque jelly in 2 hrs
2 0	1·3	1·0	0·7	Opaque jelly in 2½ hrs
2 0	1 0	0 5	0 5	Clear solution, no jelly
2·0	1·0	0 5	1 0	Translucent jelly in 15 hrs
2·0	1·0	0·5	1·3	Opaque jelly in 2½ hrs
1 0	1 0	0 5	0 7	Opaque jelly in 22 hrs
0·5	1·0	0 5	0·7	Loose jelly in 24 hrs

By studying the influence of the variation of the concentration of these reactants, it has been observed that as

in the case of ferric hydroxide, a minimum amount of sodium acetate is necessary for a given amount of chromic chloride for the jelly formation Similarly, the addition of greater quantities of ammonium sulphate always gives the jellies of weaker texture in a longer time. The regulation of the quantities of ammonia is also an important factor in the formation of this jelly , and a sufficient quantity of ammonia (which is much greater than was necessary for the preparation of ferric hydroxide jelly) has always to be added before a jelly could be expected

The jellies obtained by dilute solutions of chromic chloride are translucent, but those with concentrated solutions are opaque The jellies are very stable and of fine texture, and do not undergo any marked syneresis

The jelly prepared by Weiser's method (loc cit) by the addition of the excess of caustic alkali to chromic chloride solution is green and not so fine in texture as obtained by our method The jellies which we have described are violet in colour and resemble those of Reinitzer, though we have prepared them at the ordinary room temperature

Aluminium Hydroxide Jelly —Not much work appears to have been done on this jelly A sol formed by peptising sufficient amount of hydrous alumina to form a viscous liquid has been observed to set to a jelly on standing. The jelly breaks up on shaking and cannot be re-converted to the gel form. Schalek and Szegvary[1] prepared a sol by Crum's method which set to a jelly on the addition of a suitable amount of electrolyte just below the precipitation value On shaking, the sol was re-formed which again set to a jelly on standing, thus exhibiting thixotropy. It has also been observed that a jelly may be formed by peptising hydrous alumina with acetic acid but shaking converts the jelly into a gelatinous precipitate that is not re-peptised

[1] Kolloid-Z , *33*, 326 (1923)

The preparation of this jelly by the usual Prakash and Dhar's method[1] requires more regulation of the concentrations of the reactants than iron or chromium hydroxide jellies To M/2 solution of aluminium nitrate are added varying concentrations of 3 54 N sodium acetate and 2M ammonium sulphate, and then a little of 5 81 N ammonia is added, drop by drop with constant stirring, and thus a clear colourless solution is obtained which soon develops opalescence on standing, and if the concentrations are favourable, firm translucent or opaque jellies are obtained

In some cases, the opalescence of these jellies increases with time and finally even translocent jellies become opaque. Some of the concentrations for the preparation of these jellies are given below

TABLE III

Total Volume—5 c c

M/2 $Al(NO_3)_3$	3 54N Sodium acetate	2M Ammonium sulphate	5 81N Ammonia	Observation
c. c.	c. c	c c	c c	
2·0	1·0	0 5	0 5	Transparent jelly in 3 days
2 0	1 0	0 7	0 5	Translucent jelly within 22 hrs
2 0	1·2	0 7	0 5	Precipitate, no jelly
2·0	1 0	1·0	0 5	Translucent jelly in 23 hrs
2·0	1 0	1 2	0 5	Opaque jelly in 22 hrs
2 0	1·0	0 5	0 6	Translucent jelly in 27 hrs
2 0	1·0	0 5	0 7	White precipitate, no jelly
2 0	1 0	0·7	0 3	Transparent jelly in 3 days
2 0	1·0	0 7	0 4	Transparent jelly in 26 hrs
1·0	1·0	0·7	0·5	No jelly
2(of 0 75M)	1·1	0 7	0 5	Translucent jelly in 2 days
2(of 0·75M)	1 1	0·7	0 6	Precipitate, no jelly

[1] Loc cit

There appears to be a very limited range over which the quantity of sodium acetate could be varied. The addition of large amounts of ammonium sulphate slightly decreases the time of setting, but increases the opacity of the jelly. It has also been observed that greater the concentration of ammonium sulphate, the less would be the amount of ammonia necessary to give a jelly The addition of ammonia in large quantities, however, gives either opaque or loose jellies or precipitates Aluminium hydroxide jellies prepared by our method are very stable, quite uniform in texture.

Stannic Hydroxide Jelly—It has been observed[1] that when a colloidal solution of hydrous stannic oxide is evaporated, a transparent jelly is obtained, whilst precipitation with electrolytes is said to give always a gelatinous precipitate, but no jelly. Weiser[2] prepared colloidal stannic oxide by Zsigmondy's method, *i.e.*, by allowing a small amount of stannic chloride-hydrate to stand in a large amount of water for three days, and washing the resulted hydroxide by the aid of centrifuge until it was so free from chlorides that it started to go into the colloidal solution Several of these washed portions were combined, shaken up with water containing a small amount of ammonia, and allowed to stand until the peptisation was complete. The excess of ammonia was removed by boiling which ages the colloidal oxide The sol obtained in this way was mixed with different amounts of coagulating electrolytes, and allowed to stand for two days Under suitable conditions, this gave transparent jellies, and sometimes only cloudy jellies could be obtained.

We have prepared stannic hydroxide jellies by the addition of varying concentrations of 3·54 N sodium acetate to M/2 solution of tin tetrachloride (liquid Kahlbaum) in

[1] Zsigmondy Spear, "Chemistry of Colloids," 155 (1917).
[2] J Phys Chem., *26*, 681 (1922).

presence of small quantities of ammonium sulphate. The solution soon develops opalescence and finally a jelly is obtained The addition of ammonia is not necessary to obtain this jelly

In certain cases, it has been observed that where stannic chloride solution is accompanied with free hydrochloric acid as in the cases of hydrated crystals of stannic chloride, or an old solution of tin tetrachloride, the addition of ammonia is also essential to obtain a jelly. Some of the concentrations to give a jelly are given below.

TABLE IV

Total Volume—5 c c

M/2 $SnCl_4$	3·54% Sodium acetate	2% Ammonium sulphate	58% Ammonia	Observation
c. c	c c	c c	c c	
2·0	0·7	0·5	0	Clear solution, no jelly.
2·0	0·9	0·5	0	Opaque jelly in one day.
2·0	1·0	0·5	0	Opaque jelly in one day, slight syneresis after two days
2·0	1·2	0·5	0	Immediately opaque jelly, readily undergoing syneresis
2·0	1·0	0·6	0	Opaque jelly in 14 minutes, syneresis after 5 hrs
2·0	1·0	0·7	0	Opaque jelly in 10 minutes
2·0	1·0	0·9	0	Opaque jelly in 2 minutes, syneresis soon starts.
2·0	0·7	0·5	0·1	White opaque jelly in 32 hrs
2·0	0·7	0·5	0·2	White opaque jelly in 22 hrs
2·0	0·7	0·5	0·3	Loose jelly immediately, soon undergoing syneresis
1·0	0·5	0·1	0	Opaque jelly in 10 minutes
1·0	0·4	0·1	0	Firm opaque jelly in one day.

It has been observed that there is always a limited range over which the quantity of sodium acetate can be extended to give a jelly The jellies obtained by the addition of large amounts of either sodium acetate or ammonium sulphate begin to break or synerise at once The addition of ammonia is also necessary where the concentrations of sodium acetate and ammonium sulphate are insufficient to give jellies

Stannic hydroxide jellies obtained by the above method are opaque. Some of these are very stable, while others break up on ageing

Zirconium Hydroxide Jellies.—It was, perhaps, for the first time observed by Rosenheim and Heitzmann,[1] and afterwards by Dhar and collaboratore[2] that zirconium hydroxide jellies can be obtained by the dialysis zirconia sols. If 10 per cent solution of zirconium nitrate be allowed to dialyse for about a week, a clear sol is obtained which when coagulated with potassium chloride or sulphate yields jellies or gelatinous precipitates according to the conditions. The jellies are perfectly transparent, and if the electrolyte added is not in too much excess, the jellies may be kept as such for months without undergoing marked syneresis If the coagulating electrolyte is added in excess, the jellies rapidly synerise

I have further observed that unstable translucent jellies of zirconium hydroxide can be obtained by simply adding sodium acetate to zirconium nitrate solution and allowing the mixture to stand for a few minutes The addition of sulphate ions is not essential, though favourable for the formation of the jellies as has been shown in the following table :—

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[1] Ber., *40*, 810 (1907)

[2] J. Indian Chem. Soc , *5*, 309 (1928)

and Dhar[1] have studied the nature of the syneresis of this jelly

Scandium Oxide Jellies—This jelly has not attracted much attention, simply Bohm and Niclassen[2] made some observations with it. By dialysing a solution of scandium chloride, $ScCl_3$, to which ammonia is added short of precipitation, a hydrous sol results which sets to a jelly when treated with suitable amounts of electrolytes It appears that this jelley also exhibits thixotropic property, for on shaking under favourable conditions, it is transformed to a sol condition, and again reversed to a gel state on standing quietly

Erbium Oxide Jelly—Bohm and Niclassen[3] used the method of the preparation of scandium oxide jelly in the preparation of orbium oxide jelly They dialysed a solution of erbium nitrate to which ammonia was added short of precipitation The sol thus obtained sets to a jelly on adding a suitable amount of coagulating electrolytes

Ceric Hydroxide Jelly—Perhaps, Biltz[4] was the first to obtain this jelly, but Fernau and Pauli[5] were the first to make an important investigation of the various properties of the sol and they also observed that β and γ rays from radiumact on it in much the same manner as coagulating electrolytes The sol on the prolonged exposure gives the jelly.

Ceric hydroxide jelles are best prepared by coagulating the sol obtained by the dialysis of a 10 per cent ceric ammonium nitrate for about 5—7 days Kruyt and van der Made[6] observed that if the dialysis be carried to sufficient extent, the sol sets itself to a firm jelly. This jelly returns to the sol condition if shaken up with a quantity

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[1] J. Indian Chem Soc , 7, 417 (1930)
[2] Z anorg Chem , *132*, 6 (1924)
[3] Loc. cit
[4] Ber , *35*, 4435 (1902), Z anorg Chem., *168*, 96 (1927)
[5] Kolloid-Z , *20*, 20 (1917)
[6] Rec. Trav. Chim , (4) *42* 277 (1923)

of freshly dialysed sol Nitric acid is undoubtedly a peptising agent in the sol, and thus the sol is more stabilised in its presence Dhar[1] farther studied this jelly. It appears that the temperature at which the dialysis is carried has much influence upon the gelation properties of this substance. The higher the temperature of dialysis, the less is the hydration tendency developed by the particles I have observed that if ceric ammonium nitrate be dialysed at the temperature of tropical summer, the sol yields jellies only with difficulty. The jellies are more readily formed if the coagulation is affected by iodide ions than with chloride or nitrate

Ceric hydroxide gives transparent jellies, some of which are very stable and can be preserved as such whilst others undergo marked syneresis in the course of time Certainly, this depends on the purity of the sol and the concentration of the coagulating electrolyte used in the preparation of the jelly.

Desai[2] has observed that the time required for the gel formation in the dialyser decreases considerably and the degree of the hydration of the gel increases with the rise in temperature at which the dialysis is carried out However, I am of the opinion, that as the temperature increases the degree of hydration must decrease provided the other factors are the same In the experiments of Desai, the apparent increase in hydration is not directly due to the increase in temperature, but to the fact that at higher temperatures, the process of dialysis is quicker and thus the sol is more readily purified, and certainly, the greater the purity of the sol, the less would be the time of gelation.

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[1] Chakravarti, Ghosh and Dhar, Z. anorg. Chem., *164*, 63 (1927)

[2] Kolloid, Chem Beih., *26*, 422 (1928).

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[1] J. Indian Chem. Soc., 7, 417 (1930).
[2] Z. anorg. Chem., *132*, 0 (1924).
[3] Loc. cit.
[4] Ber., *35*, 4435 (1902), Z. anorg. Chem. *168*, 90 (1927).
[5] Kolloid-Z., *20*, 20 (1917).
[6] Rec. Trav. Chim., (4) *42* 277 (1923).

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[1] Chakravarti, Ghosh and Dhar, Z. anorg. Chem., *164*, 63 (1927)

[2] Kolloid. Chem Beih., *26*, 422 (1928).

Mercuric Oxide Jelly—Reynolds[1] was the first to observe that mercuric oxide is capable of giving jellies in non-aqueous medium, and this jelly was further studied by Bunce[2] in details They observed that by adding mercuric chloride to a normal solution of KOH containing 40 c c. of acetone, a sol is obtained which sets to a firm jelly on standing, the time required depending upon the concentration of the sol

The easiest method, according to Bunce, is to dissolve 30 gms caustic potash and 20 c c acetone in 500 c c. water and to add slowly a saturated solution of mercuric chloride The mixture is continuously shaken, till the appearing precipitate goes on dissolving, and the first faint permanent precipitate occure The mixture is allowed to stand for some hours. After a short time white opalescence develops, and finally, if the concentrations are favourable, white solid opaque jelly is obtained. In some cases, jellies with permanent supernatent liquid are obtained.

Bunce has further observed that the addition of potassium sulphate or sodium nitrate has no apparent effect on the gelation Addition of potassium carbonate caused the formation of a viscous milky liquid, while cobalt sulphate or copper nitrate caused the formation of a granular precipitate With sodium acetate, a jelly-like structure was obtained but not a real jelly

A slight rise in the temperature causes a mixture to gel more quickly, but heating for 5 minutes or more at temperatures above 63° seems to prevent the formation of jellies Bunce observed that it is impossible to get a jelly if the mercuric chloride is originally contaminated with a mercurous salt.

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[1] Proc. Roy. Soc , *19*, 431 (1871).

[2] J. Phys Chem., *18*, 269 (1914)

Magnesium Hydroxide Jelly.—Recently, Kroger and Fischer[1] have reported the formation of magnesium hydroxide jelly. It has been prepared by adding water to a 3 per cent solution of magnesium ethoxide in methyl alcohol. Such a gel is not very stable and rapidly undergoes syneresis, the more readily the higher the concentration. The gel may be stabilised by the addition of glycerol, glycol, etc., due to the peptising effect. By using water, glycerol and alcohol in proportions of 5 . 10 · 10, a plastic glass clear gel which is stable for months can be prepared. Mixed gels have also been obtained.

Nickel Oxide Jelly.—Tower[2] has described the preparation of this jelly by dissolving nickel acetate in glycerol to which an alcoholic solution of caustic potash was added. A green gel was obtained by this method, which on standing undergoes syneresis, but the dialysis of this sol once more formed a gel on removal of KOH.

Another method for the preparation of the jelly consists in mixing equivalent concentrations of nickel tartrate and caustic potash. When the solutions are as concentrated as normal, precipitation takes place slowly, giving a transparent green jelly.

Arsenate Jellies

Some of the arsenate jellies rank amongst the best in the whole of our literature. The metals which could successfully give arsenate jellies are manganese, zinc, iron, chromium, thorium, tin and cerium.

Manganese Arsenate Jellies.—Deiss[3] has claimed for the priority in the case of this discovery. The jellies are prepared by mixing manganous chloride and potassium arsenate (KH_2AsO_4) solutions in right proportions. Deiss observed that the jellies thus prepared are very stable and can usually

[1] Kolloid-Z., *17*, 5 (1920).
[2] J. Phys. Chem., *26*, 733 (1922).
[3] Kolloid-Z., *14*, 139 (1914); *15*, 16 (1915). Z anorg. Chem., *116* 228 (1921).

be kept for weeks without appreciable change. Sooner or later, however, rose-coloured crystals begin to separate. Klemp and Gyulai[1] observed that by the successive addition of ammonium sulphate, acetic acid and excess of sodium arsenate to solutions of zinc, ferrous, manganous, cobalt, cadmium and calcium salts, the colloidal solutions of the arsenates of these metals are obtained in the form of opalescent jellies. Crystals begin to separate from these jellies when kept for some time. In the absence of acetic acid and ammonium sulphate, these solutions yielded only gelatinous precipitates, but no jellies

The further work on this jelly is of Kraemer[2] and Weiser[3] Kraemer obtained this jelly by the addition of manganous sulphate to the solution of potassium arsenate He studied the effect of various anions and cations on its gelation He observed that the lowering of the temperature favours the jelly formation The time of gelation of this jelly cannot be much extended beyond 10—15 seconds A slight warming of the solutions always hastens up the beginning of the gelation. It appears that the rise of temperature is necessary to start up the process of jelly formation.

Zinc Arsenate Jellies—Manganese and zinc arsenate jellies are indistinguishable. Both are perfectly transparent and stable. Sometimes they undergo slight syneresis. Zinc arsenate jellies were for the first time prepared by Klemp and Gyulai.[4] They have obtained this jelly by the addition of potassium dihydrogen arsenate to a solution of zinc sulphate. According to them, disodium or trisodium arsenates, Na_2HAsO_4 or Na_3AsO_4, if previously neutralised by the addition of hydrochloric or acetic acid also yield

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[1] Ibid, *15*, 202 (1914)
[2] Colloid Symp Mono. Wisconsin, *1*, 62 (1923)
[3] J. Phys Chem, *28*, 26 (1924)
[4] Kolloid-Z, *22*, 57 (1918)

jellies. Crystals appear to separate out of these jellies after two or three months Weisor[1] has also made some experiments on it.

Ferric Arsenate Jellies.—Manganese and zinc arsenate jellies were prepared by the metathetical reactions of the two salts. However, the method could not be successfully employed in the case of other jellies. The credit of the preparation of excellent jellies of ferric and chromic arsenates goes to Holmes and his co-workers However, Grimaux[2] was the first to obtain this jelly Holmes and Arnold[3] observed that precipitated ferric arsenate is readily peptised by ferric chloride, ferric sulphate, or ferric nitrate. On dialysis, these colloids yield gels of excellent clearness and texture, except in the case of ferric sulphate, whereby a powdery coagulam is obtained

The best method of the preparation of this gel is to coagulate the sol obtained by dialysing a mixture of ferric chloride in excess and potassium arsenate. Potassium arsenate when added to a solution of ferric chloride gives a yellowish white precipitate which dissolves on shaking, in the presence of the excess of ferric chloride. The addition of potassium arsenate is stopped when about three-quarters of the ferric chloride has been transformed to the arsenate. The solution at this stage is faint yellow in colour. The mixture is now allowed to dialyse for about a week. It gradually develops red colour as the process of dialysis proceeds on, which Holmes and Arnold rightly think to be due to the formation of ferric hydroxide sol by the hydrolysis of a little quantity of ferric chloride which was present there in excess The sol, purified by dialysis, gives excellent transparent and stable jellies on the addition of electrolytes like potassium chloride or sulphate. The sol

[1] Loc. cit.

[2] Compt Rend., *98*, 1540 (1884)

[3] J. Amer. Chem. Soc., *40*, 1014 (1918)

is positively charged and not negative as Holmes[1] was led to think. If the dialysis is continued for a long time, the sol sets to a transparent jelly in the parchment dialyser Highly purified sols set themselves to jellies on ageing without the addition of foreign coagulating electrolytes If the ferric chloride is not sufficient to peptise the whole of arsenate and still to remain in excess, the curdy or opalescent jellies are obtained

Chromic Arsenate Jellies—Holmes[2] and co-workers have also prepared chromic arsenate jellies The method of the preparation is exactly the same as was used in the case of ferric arsenate A mixture of chromic chloride in excess and potassium arsenate ($KH_2 As O_4$) is dialysed for about a week and the clear greenish sol is coagulated by the addition of potassium chloride or sulphate, whereupon a clear transparent greenish gel is obtained. The jelly is very stable and can be preserved without undergoing any change In the course of time, it acquires the vibrating property, and so does ferric arsenate jelly too When sufficiently purified by dialysis, the sol sets to the jelly in the dialyser itself or in the bottle when allowed to age without the extra addition of coagulating electrolytes

Mention has been made by Weiser[3] of the preparation of other arsenate jellies of cadmium, cobalt, aluminium, ferrous, etc, but the results are not much encouraging, and the jellies obtained are not fine in texture

Thorium Arsenate Jellies.—Prakash and Dhar[4] have obtained for the first time the jellies of thorium arsenate These jellies resemble manganese and zinc arsenates in their mode of preparation but differ from them in being slightly turbid and also in the fact that they require much higher concentration of arsenate solutions for the preparation

[1] J. Amer Chem Soc, *38*, 1972 (1916)
[2] Loc. cit.
[3] J. Phys Chem, *28*, 26 (1924)
[4] J. Indian Chem. Soc., *6*, 587 (1929)

When to a thorium nitrate solution, a few drops of potassium arsenate solution are added a gelatinous precipitate appears which rapidly dissolves on shaking in the presence of an excess of thorium nitrate solution The solution develops viscosity and finally the whole mass sets to an opalescent jelly. The best jellies of thorium arsenate are prepared by taking 5 c c of a solution of thorium nitrate (12·035 gms in 250 c.c) and adding to it 0 2 c c. to 0·4 c.c. of 18 per cent potassium arsenate solution raised to 1 c c. The mixture is shaken for about 2 minutes, and then allowed to set. The time of setting can be extended from that of a few minutes to about 24 hours by varying the concentrations of potassium arsenate

These jellies are almost transparent with slight opalescence. In some cases the opalescence increases with time and the jellies ultimately become translucent or opaque. The jellies are very stable and do not undergo any syneresis.

Stannic Arsenate Jellies.—This jelly has also been for the first time prepared by Prakash and Dhar.[1] Stannic chloride solution when mixed with potassium arsenate solution gives the precipitate of stannic arsenate, but if stannic chloride be in excess, this precipitate dissolves and a clear colourless solution is obtained which on keeping develops opalescence and finally sets to an opalescent jelly on standing for some time.

Stannic arsenate jellies are opalescent or translucent at the time of formation, but they become opaque afterwards. The opacity increases more rapidly with the concentration of the potassium arsenate solution. However, the jellies are very stable, and do not undergo any marked syneresis.

The best stannic arsenate jellies are obtained by mixing 3 c.c. of M/1·099 stannic chloride solution with

[1] Loc. cit.

which gives transparent stable jellies on coagulation with electrolytes as potassium chloride or sulphate The sol when highly purified by dialysis also yields jellies on ageing by itself without adding any electrolyte

Holmes and Arnold[1] have observed that a gel originating from the diammonium hydrogen phosphate (in a series of unwashed precipitates) sets in three days, the gel from the disodium salt in eight days and as might have been expected, that from a combination of these two, sodium ammonium hydrogen phosphate, in intermediate time, say five days. However, the best gels are obtained by the use of potassium dihydrogen phosphate.

Chromium and Aluminium Phosphate Jellies.—Holmes and Rindfusz (loc. cit) have observed that similar to ferric phosphate jellies, aluminium and chromium phosphate jellies can also be prepared by coagulating their sols obtained by the dialysis of the mixtures of their chlorides or nitrates and potassium dihydrogen phosphate However, not much work has been done on these jellies. It appears that aluminium and chromium phosphates have less tendency of developing hydration, and consequently, their jellies are not so readily prepared as in the case of ferric phosphate.

Thorium Phosphate Jellies.—These jellies have been for the first time prepared by Prakash and Dhar[2] They are prepared with the same ease as zinc and manganese arsenate jellies, and are amongst the most beautiful of the jellies so far prepared They are perfectly transparent and free from opalescence, and as stable as could be kept as such for months without apparently undergoing any change or syneresis The jellies are of the best texture and markedly elastic The time of gelation in their case can easily be extended over a very long period by regulating

[1] J. Amer Chem Soc., *40*, 1014 (1918)
[2] J. Indian Chem. Soc , *6*, 587 (1929)

the concentrations of the reactants. These are the first phosphate jellies which had been prepared metathetically.

5 c c of a solution of thorium nitrate (12·035 gms. in 250 c c) are taken in test tubes and varying amounts of 22 per cent potassium phosphate solution (about 0·2 to 0·4 c c.) are added, keeping the final volume 6 c.c. The mixtures are shaken well for about 3 minutes and then allowed to stand. The time of the setting of the jellies depends upon the concentration of potassium phosphate used By regulating its concentration, the time of gelation can be extended from that of a few minutes to that of three days Thorium phosphate jellies are so stable that they do not break or syneriso in even months and may be preserved for over a year

Stannic Phosphate Jellies—This jelly has also been prepared for the first time by Prakash and Dhar [1] It is prepared exactly in the same way as stannic arsenate jellies with which it resembles in every respect The best stannic phosphate jellies are obtained by adding 1 to 3 c.c. of 22 per cent potassium phosphate solution (KH_2PO_4) to 3 c c of M/1 099 stannic chloride solution, and making the total volume 6 c.c. The transparent mixture so obtained develops opalescence and finally translucent or opaque jellies are obtained

These jellies are also very stable and exhibit no marked syneresis The opacity of these jellies increases on standing and finally, all the jellies become completely opaque

Molybdate Jellies

No molybdate jellies had been previously prepared before we undertook the work Prakash and Dhar[1] have for the first time prepared the molybdate jellies of iron, thorium, tin and zirconium.

Ferric Molybdate Jellies—When potassium molybdate solution is added to a ferric chloride solution, a yellowish white precipitate is obtained which dissolves on shaking if

[1] J Indian Chem. Soc., *6*, 587 (1929), *7*, 367 (1930)

ferric chloride is in excess. The clear mixture on standing for some time develops opalescence and finally, if the concentrations are suitable, the whole mixture sets to a firm opaque jelly

Ferric molybdate jellies are obtained by adding varying amounts (3·5 to 5 c c) of 10 per cent potassium molybdate solution to 4 c c of M/2 69 ferric chloride solution, keeping the total volume to be 10 c c The clear mixture obtained by well-shaking the constituents sets to firm opaque jelly within a day or so

Thorium Molybdate Jellies —When potassium molybdate solution is added to thorium nitrate, a white precipitate of thorium molybdate occurs, but if the mixture is vigorously shaken, the precipitate goes on dissolving till a clear viscous solution is obtained This mixture on standing for some time, sets to a transparent colourless jelly. These jellies are very stable and do not synerise The best of the thorium molybdate jellies are prepared by adding about 0 3 to 0·8 c.c. of 10 per cent potassium molybdate solution to 5 c c. of thorium nitrate solution (12 035 gms salt in 250 c c) making the total volume 6 c.c., and allowing the mixture to stand for 5 minutes or so Some of the molybdate jellies prepared by the use of comparatively higher concentrations of potassium molybdate break up in the course of 10—12 days leaving a white powder

Stannic Molybdate Jellies.—No jelly has been obtained by directly mixing the solutions of potassium molybdate and stannic chloride whereby a white precipitate of gelatinous nature is only formed. However, if potassium molybdate (15 per cent solution) be added below precipitation value to stannic chloride solution and the mixture is allowed to dialyse for 24 hours, a clear sol with slight opalescence is obtained The sol is fairly stable and sets to translucent firm jellies on the addition of coagulating electrolytes like potassium chloride or sulphate. Like other stannic jellies,

these are almost transparent though accompanied with slight opalescence, at the time of formation, but become opaque on keeping for some time

Zirconium Molybdate Jellies—When potassium molybdate solution is added to zirconium nitrate solution, a white precipitate of zirconium molybdate is obtained which is easily dissolved by an excess of zirconium nitrate on shaking In this way, a sufficient amount of zirconium molybdate can be peptised and a concentrated sol obtained

This sol on dialysis gives suitable jellies when coagulated with electrolytes like potassium chloride or sulphate If the dialysis were carried for a long time, the sol either sets on the parchment paper or gives a transparent jelly on standing for some days without the addition of electrolytes

Some of the jellies prepared by the coagulation of the sol by potassium chloride develop opalescence and may even become opaque

A sol prepared by adding 10 per cent solution of potassium molybdate to 70 c c of M/1 33 zirconium nitrate till the precipitate obtained just dissolved in excess of zirconium nitrate and dialysed for 36 hours gave good jellies with N-KCl or N/5 potassium sulphate Strength of the sol was 50 3 gm zirconium molybdate per litre

Tungstate Jellies

No tungstate jelly has ever been prepared before Prakash and Dhar[1] have prepared ferric tungstate, chromic tungstate, stannic tungstate and thorium tungstate jellies No jellies have yet been prepared of the tungstate of ceric or zirconium.

Ferric Tungstate Jellies—Ferric tungstate jellies have been obtained by two methods firstly, by directly mixing ferric chloride with sodium tungstate, and secondly, by dialysing and coagulating the sol obtained by peptising ferric tungstate with excess of ferric chloride

[1] J Indian Chem Soc, Loc cit.

When 15 per cent solution of sodium tungstate is added to M/2 ferric chloride, a bulky precipitate is formed which dissolves to some extent on vigorous shaking If the mixture be warmed in a water-bath at 96°C for 5 minutes, it is completely dissolved, and a clear transparent yellow solution is obtained which sets to fine yellow opaque jelly when the concentrations are suitable.

A yellow opaque jelly is obtained by mixing 2 c c of M/2 ferric chloride with 3 c.c of 15 per cent sodium tungstate, and warming the mixture for 5 minutes at 95°C These jellies resemble ferric molybdate jellies and are very stable.

More transparent jellies are obtained when a mixture of sodium tungstate and ferric chloride *in excess is* dialysed and the sol thus obtained is coagulated with electrolytes. To 75 c c of 0 929 M ferric chloride solution was added a solution of 16 5 gms sodium tongstate (Na_2WO_4. $2H_2O$) with vigorous shaking and slight warming and the volume was made up to a litre The mixture was filtered ond diolysed for six days The clear sol thus obtained contained 21 68 gms. ferric tungstate per litre and set to translucent jellies when coagulated with N-KCl or N/20 K_2SO_4.

Chromic Tungstate Jellies —When sodium tungstate solution is added to chromic chloride solution, a bulky greenish white precipitate is formed, which slightly dissolves on shaking at the room temperature (25°—30°C) oven when chromic chloride is present in large excess. However, if the mixture is warmed and shaken, a sufficient amount of chromic tungstate is peptised and it forms a clear transparent solution If this mixture is now allowed to dialyse for about ten days, a translucent sol is obtained which sets to a jelly on the addition of potassium sulphate.

To M/1 5 chromic chloride solution, a 15 per cent solution of sodium tungstate was added, so long as the precipitate obtained could be re-dissolved on warming. The

of stannic chloride was dialysed for 24 hours A clear transparent sol containing 66·4 gms. SnO_2 per litre was obtained which set to transparent jellies with slight opalescence when coagulated by the addition of electrolytes, potassium chloride or sulphate The sol itself becomes more and more viscous even without the addition of electrolytes and sets within 24 hours

Zirconium Borate Jellies —Zirconium borate jellies are obtained in the same way as zirconium molybdate jellies A hot concentrated solution of borax was added to 70 c c of M/1·33 zirconium nitrate solution till the precipitate of zirconium borate obtained just dissolved in the excess of zirconium nitrate The solution was dialysed for three days The concentration of the sol thus obtained was 34·62 gms zirconium borate per litre It gave transparent or opalescent jellies when coagulated by electrolytes, like N-KCl or N/5 K_2SO_4

It has been observed in the case of both zirconium molybdate and borate jellies that they are readily obtained when their sols are coagulated by potassium chloride but these jellies develop opalescence on standing However, when the sol is coagulated by potassium sulphate, the jellies are more transparent and do not develop opalescence The jellies are very stable, and if the coagulating electrolyte is not in much excess, they do not undergo any marked syneresis

Ceric Borate Jellies —When a solution of borax is added to a solution of ceric ammonium nitrate, a yellowish white is obtained which readily dissolves on shaking, if the ceric ammonium nitrate is in excess. If the mixture of the two substances is allowed to stand for some time, the contents are generally precipitated, though in some cases loose unstable jellies may also form

The clear solution formed by mixing 100 c c of 10 per cent ceric ammonium nitrate and 35 c c of 15 per cent

borax solution was dialysed for 24 hours The sol thus obtained not only gave a jelly on treatment with N/20 potassium sulphate solution, but also on keeping for some time in a jena glass bottle, its viscosity increased continuously and in the next 24 hours, it set completely to an opalescent jelly.

Another sol was prepared by mixing only 30 c c of 15 per cent borax solution to 100 c c of 10 per cent ceric ammonium nitrate. The mixture on dialysis gave a clear sol in the course of 24 hours The sol was quite stable and gave stable jellies on the addition of electrolytes.

Sulphide Jellies

The sulphide sols are more or less hydrophobic when compared to the sols of hydrous oxides They do not appear to develop hydration tendency which is so essential for the formation of jellies Any record of the formation of sulphide jellies is of Usher,[1] who appears to have gelatinised cadmium sulphide in the presence of suitable concentrations of sodium chloride. This method he also employed for the preparation of gamboge jellies

Usher prepared a sol of cadmium sulphide by passing hydrogen sulphide through the thoroughly washed precipitate suspended in water. This sol was treated with varying quantities of sodium chloride and it was found to gelatinise if the concentration was between N/100 and N/10. Thus in a mixture in which the final concentration of cadmium sulphide was 1 51 per cent by volume, decinormal sodium chloride caused gelatinisation immediately ; twenty-fifth normal in two minutes and fiftieth normal after several hours

Von Weimarn Jellies

Von Weimarn has done a sort of pioneering work in the field of precipitation, and his laws in this connection have

[1] *Proc. Roy. Soc., A., 125, 143 (1929).*

Calcium acetate jelly.—Baskerville[1] observed that if 85 c.c. of 95 per cent alcohol be mixed with 15 c.c. of the saturated solution of calcium acetate in water the whole mass of the alcohol sets to a fine solid transparent jelly. The jelly undergoes syneresis in a short time. The alcohol of the jelly can be replaced exactly in the same manner by acetone.

Recently, Thorne and Smith[2] have studied this jelly in details. They prepared the jelly by pouring a saturated aqueous solution of calcium acetate into alcohol. Most of the gels thus prepared are not stable for more than 24 hours; they are opalescent at first but gradually soften with time. The stability of the gels is increased in some cases to six months, by the addition of acetone or various oleates. Gels containing sodium oleate exhibit syneresis. These investigators have also studied the influence of temperature, and various ions on the stability of these jellies.

[1] U. S. P. 1208265 (1916).
[2] Kolloid-Z., *48*, 113 (1929).

DETECTION OF IRON, THALLIUM, TITANIUM AND ZIRCONIUM IN A MIXTURE

BY

I K TAIMNI

The analysis of mixtures containing only common elements can be carried out without much difficulty, because as a result of extensive investigations of the analytical properties of these elements we have now at our disposal fairly satisfactory schemes for their separation and detection But the separation and detection of the rarer elements is still attended with considerable difficulty as the properties of these elements have not yet been sufficiently investigated for the elaboration of methods which are simple as well as accurate. Some useful schemes have, however, been devised for the analysis of mixtures containing both common and the rarer elements. The work of Noyes, Bray and Spear in this field is especially valuable. The scheme of qualitative analysis devised by them [J.A.C S, 29, 137 (1907) and 30, 481 (1908)] can be taken as a good basis for work in the qualitative analysis of mixtures containing the common elements as well as the more important of the rarer elements. There are, however, a few manipulative operations in their scheme which render its adoption in ordinary laboratory work difficult if not impossible. It is true that the difficulties encountered in the detection of the rarer elements are so great that the inclusion in any scheme of unusual and rather inconvenient methods may be quite justified. But the more these methods are replaced by others which are simpler and more convenient, the easier it will be to adopt such schemes in ordinary class work. Under certain circumstances even some sacrifice of analytical accuracy may be justified, where, for instance, an extremely sensitive but

inconvenient method for detecting an element is replaced by a less sensitive but more convenient method A manipulative operation in the scheme put forward by Noyes, Bray and Spears which makes it unsuitable for ordinary class work is the separation of iron and thallium from zirconium and titanium by means of ether At one stage in the analysis these four metals are precipitated together in the form of hydroxides basic acetates, or phosphates The precipitate is dissolved in HCl of a definite concentration and the solution is shaken with ether in a separating funnel The chlorides of iron and thallium pass into the ethereal layer, while all of the zirconium and titanium remains in the aqueous layer. By repeating the operation twice or thrice all the iron and thallium can be dissolved out from the aqueous solution As far as the effectiveness of the operation is concerned it is undoubtedly an excellent method of separating these metals, but it needs hardly be pointed out that the method is neither cheap nor convenient, especially in the hot weather of India where the temperature in most places is above 100°F For these reasons the author had been for some time trying to devise a method of detecting these four metals in the mixed precipitate without the use of ether On studying the methods of identifying these four elements it was found that the very scheme given by the authors could be modified in such a manner as to eliminate the use of ether without necessitating any sacrifice of analytical accuracy. In the method recommended by Noyes, after the separation of iron and thallium from zirconium and titanium, thallium is identified by precipitation as thallous iodide with potassium iodide and sulphurous acid iron by the usual thiocyanate test, zirconium by precipitation as phosphate with sodium phosphate in presence of sulphuric acid, and titanium by conversion into salt of TiO_2 with H_2O_2 in presence of sulphuric acid.

Now, if the specific test for each of the four elements can be applied in presence of the remaining three

it will obviously simplify the whole procedure if the mixed precipitate were dissolved in sulphuric acid and small portions of the solution so obtained were tested for each of the four elements that may be present A study of these tests showed that it is possible to test for each of the elements in a portion of the solution obtained with sulphuric acid without any interference from the other elements provided the concentration of sulphuric acid is properly regulated Before dealing with the procedure to be adopted the individual tests may be discussed

(i) *The Test for Thallium* —In order to determine the sensitiveness of the test for thallium with potassium iodide and sulphurous acid, a solution of thallic chloride (containing 0 1 mgm thallium per cc of the solution) was added from a burette to mixtures of potassium iodide and sulphurous acid containing varying quantities of sulphuric acid, and the points at which distinct precipitate of thallous iodide appeared were determined The following results were obtained.

TABLE I

Showing the effect of varying the concentration of H_2SO_4 on precipitation of TlI

1N KI=1 cc
0 6N H_2SO_3=5 cc
H_2SO_4=10 cc
Total volume=20 cc

	10N H_2SO_4	5N H_2SO_4	1N H_2SO_4	0N H_2SO_4
05 mgm Tl	No precipitate	No precipitate	No precipitate	No precipitate
0·1 mgm. Tl	No precipitate	No precipitate	No precipitate	No precipitate
0 2 mgm Tl	Opalescence	Opalescence	Opalescence	Opalescence
0 3 mgm. Tl	Distinct precipitate	Distinct precipitate	Distinct precipitate	Distinct precipitate

From the table given above it will be seen that 0 1 mgm Tl can be detected in a volume of 10 cc. and the quantity of H_2SO_4 up to a concentration of 5N has no appreciable effect on the sensitiveness of the test Since the other three metals do not give a precipitate with KI in presence of sulphuric acid, the presence of thallium can be detected by taking a small portion of the solution in sulphuric acid and treating it with KI and H_2SO_3 A test analysis with 100 mgm each of Ferric iron, zirconium and titanium and 0 1 mgm of Tl showed that a small amount of thallium can be easily detected in presence of a large excess of the other metals (In the test analyses the metals were *precipitated together as hydroxides with NH_4OH, the precipitate* was dissolved in sulphuric acid and the solution tested for the metal present in small quantity by the specific test)

(ii) *The Test for Iron*—The delicacy of the thiocyanate test for iron is well known Even 0 01 mgm Fe in 10 cc. of the solution can be easily detected Since the other three metals do not give any colour or precipitate in presence of sulphuric acid, a portion of the solution in sulphuric acid can be tested for iron by means of potassium thiocyanate Of course, if it is necessary to add ferric chloride for the separation of phosphate from metals of the alkaline earth group, the test for iron should be performed before the solution is treated with ferric chloride solution It may be mentioned here that on the addition of a potassium thiocyanate to a solution containing thallic salt and sulphuric acid a yellow colour appears but this colour fades very quickly and there is no difficulty in detecting the presence of iron. A test analysis with 100 mgm each of thallium, titanium and zirconium and 0·1 mgm iron showed that it is easy to detect even a trace of iron in presence of a large excess of the other metals

(iii) *The Test for Titanium*—It might be imagined that the presence of iron in the mixed precipitate will hinder

the detection of small quantities of titanium on account of the yellow colour of ferric salts As a matter of fact, it is not at all difficult to detect even a trace of titanium in presence of a large quantity of ferric salt because dilute ferric sulphate solutions in presence of sulphuric acid are practically colourless and no colour is developed even on treating the solutions with H_2O_2 If, therefore, the mixed precipitate of the hydroxides is dissolved in sulphuric acid and a portion treated with H_2O_2 even a trace of titanium will be easily detected by the appearance of a yellow colour Should the solution before the addition of H_2O_2 have a slightly yellow colour, owing to the presence of a very large quantity of iron, all that is necessary is to dilute a portion of the solution sufficiently, so that the colour of ferric salt is almost inappreciable As the H_2O_2 test for titanium is extremely delicate this dilution does not in any way hinder the detection of even small quantities of titanium. A test analysis with 100 mgm each of iron, zirconium and thallium and 0·1 mgm titanium showed that quantities of this element even smaller than 0·1 mgm can be easily detected in presence of a large quantity of iron An additional blank test was performed with 100 mgm. iron and H_2O_2 in presence of sulphuric acid, when no change in colour was observed. In view of the extremely delicate nature of the H_2O_2 test for titanium it appears superfluous to precipitate the titanium again as phosphate by adding sodium phosphate to the acid solutions. The phosphate test is decidedly less delicate and less distinctive on account of the similar precipitation of zirconium phosphate. The presence of a large excess of sulphuric acid does not interfere with the H_2O_2 test while it does hinder the precipitation of titanium as phosphate.

(ii) *The Test for Zirconium.*—The precipitation of the phosphate in presence of a large excess of sulphuric acid is at present the most characteristic and reliable test for

zirconium No other metal except titanium gives a precipitate with sodium phosphate under this condition, but if the titanium is previously oxidized to the hexavalent condition by means of H_2O_2 the phosphate test is specific for zirconium According to Noyes, a large excess of sulphuric acid hinders the precipitation of small quantities of zirconium He found that when the concentration of sulphuric acid is 1·5N quantities of zirconium less than 1 mgm are precipitated after about an hour, and with a greater concentration of H_2SO_4 even larger quantities may not be precipitated Since in the quantitative estimation of zirconium by precipitation as phosphate the addition of even 20 per cent by weight of H_2SO_4 is advised (Treadwell, Analytical Chemistry, 1924, Vol II p 123), it was considered worth while to investigate the effect of varying the concentration of sulphuric acid on the precipitation of zirconium phosphate.

Table II

Showing the effect of varying the concentration of H_2SO_4 on precipitation of zirconium phosphate from cold solution.

Quantity of zirconium in solution	1 10 c.c. 10% Na_2HPO_4 10N H_2SO_4	2 10 c.c 10% Na_2HPO_4 5N H_2SO_4	3 10 c.c 10% Na_2HPO_4 2N H_2SO_4	4 10 c.c. 10% Na_2HPO_4 1N H_2SO_4	5 10 c.c. 10% Na_2HPO_4 0·5N H_2SO_4	6 10 c.c 10% Na_2HPO_4 0·1N H_2SO_4
0·1 and 0·2 mgm .	No opalescence	No opalescence.	No opalescence	No opalescence	No opalescence	No opalescence
0·3 and 0·4 mgm	Opalescence	Opalescence	Opalescence	Opalescence	Opalescence	Opalescence
0·5 mgm ..	Slight precipitate (finely divided)	Slight precipitate (finely divided)	Opalescence	Opalescence	Opalescence	Slight precipitate (finely divided)
0·6 mgm .	Distinct precipitate	Distinct precipitate.	Distinct precipitate	Opalescence	Opalescence	Distinct precipitate.
0·7—2·5 mgm ...	..	.		Opalescence	Opalescence	,
3—4 mgm	.	.	...	Precipitate (finely divided)	Opalescence	.
5 mgm ..	.	.	.		Precipitate (finely divided).	...

Table III

Showing the effect of varying the concentration of sulphuric acid on the precipitation of zirconium phosphate from hot solution

Quantity of zirconium in solution	1 10 c.c 10 % Na_2HPO_4 10N H_2SO_4	2 10 c.c 10% Na_2HPO_4 5N H_2SO_4	3 10 c.c 1a% Na_2HPO_4 2N H_2SO_4	4 10 c.c 10% Na_2HPO_4 1N H_2SO_4	5 10 c.c 10% Na_2HPO_4 0·2N H_2SO_4	6 10 c.c 10% Na_2HPO_4 0·1N H_2SO_4
0·1 mgm	No opalescence.	No opalescence	No opalescence	No opalescence.	No opalescence	No opalescence
0·2 mgm	Clear flocculent precipitate.	Clear flocculent precipitate	Clear flocculent precipitate	No opalescence	No opalescence	Slight precipitate.
0·3 mgm		"		Opalescence	Opalescence	Clear flocculent precipitate
0·4 mgm	"			Slight precipitate	Opalescence	
0·5 mgm	"		"	Clear flocculent precipitate	Opalescence	"
0·6 mgm	"	"			Opalescence	
0·7 mgm					Flocculent precipitate.	

A standard solution of zirconium oxychloride (containing 1 mgm. zirconium per c.c of solution) was added from a burette 0·1 c c. at a time, to cold mixtures of 10 c c of 10 per cent sodium phosphate and 10 c.c solutions of sulphuric acid of different concentrations The solutions were thoroughly shaken and allowed to stand for about 10 minutes after each addition of 0 1 c c zirconium solution The results are shown in Table II Since heating the solutions were found to accelerate the precipitation of zirconium phosphate, all the above experiments were repeated with this difference, that all the solutions after every addition of 0 1 c c zirconium oxychloride solution were heated to 60°—80° The results are shown in Table III

From tables II and III the following facts are apparent.

(i) A precipitate appears with much smaller quantity of zirconium when the solutions are heated than when they are allowed to remain cold. In the former case, the minimum quantity of zirconium which gives a precipitate in about 10 minutes is 0 2 mgm. while in the latter case it is 0 5 mgm

(ii) When the solution is heated the precipitate is obtained in a flocculent condition, while it is more or less finely divided and difficult to detect in small quantity, when obtained in the cold

(iii) The quantity of solphuric acid in solution does not make much difference except when the concentration of sulphuric acid lies near about 0·5 N—0 25 N It will be seen from the tables that the precipitate appears for the first time with practically the same quantity of zirconium in mixtures containing 5N, 2·5N, 1N, 0 05N H_2SO_4 but a much larger quantity of zirconium has to be added before a precipitate appears in *solutions containing* 0 5N and 0 25N H_2SO_4.

Table III

Showing the effect of varying the concentration of sulphuric acid on the precipitation of zirconium phosphate from hot solution

Quantity of zirconium in solution	1	2	3	4	5	6
0·1 mgm.	No opalescence	No opalescence	No opalescence	No precipitate	No precipitate	No precipitate
0·2 mgm.	Clear Tyndall beam	Clear Tyndall beam	Clear Tyndall beam	No precipitate	No precipitate	No precipitate
0·3 mgm.				Slight precipitate	Slight precipitate	Slight precipitate
0·4 mgm.				Slight precipitate	Slight precipitate	
0·5 mgm.				Clear Tyndall beam	Slight precipitate	
0·6 mgm.					Slight precipitate	
0·7 mgm.					Precipitate	

A standard solution of zirconium oxychloride (containing 1 mgm. zirconium per c.c. of solution) was added from a burette 0·1 c.c. at a time, to cold mixtures of 10 c.c. of 10 per cent sodium phosphate and 10 c.c. solutions of sulphuric acid of different concentrations. The solutions were thoroughly shaken and allowed to stand for about 10 minutes after each addition of 0·1 c.c. zirconium solution. The results are shown in Table II. Since heating the solutions were found to accelerate the precipitation of zirconium phosphate, all the above experiments were repeated with this difference, that all the solutions after every addition of 0·1 c.c. zirconium oxychloride solution were heated to 60°—80°. The results are shown in Table III.

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(ii) When the solution is heated the precipitate is obtained in a flocculent condition, while it is more or less finely divided and difficult to detect in small quantity, when obtained in the cold.

(iii) The quantity of sulphuric acid in solution does not make much difference except when the concentration of sulphuric acid lies near about 0·5 N—0·25 N. It will be seen from the tables that the precipitate appears for the first time with practically the same quantity of zirconium in mixtures containing 5N, 2·5N, 1N, 0·05N H_2SO_4 but a much larger quantity of zirconium has to be added before a precipitate appears in solutions containing 0·5N and 0·25N H_2SO_4.

This retarding influence of sulphuric acid at this concentration was repeatedly verified, and makes it necessary to keep the concentration above 1N, if small quantities of zirconium are to be detected.

It will be seen from the above that the phosphate test for zirconium is sufficiently sensitive for qualitative purposes even in presence of large quantities of sulphuric acid provided the solution is heated after the addition of sodium phosphate. It has already been shown by a number of authors that it is necessary to add a large excess of a soluble phosphate to completely precipitate small quantities of zirconium. Since the red compound formed by titanium and H_2O_2 does not decompose in presence of a large excess of sulphuric acid or on heating to 60° -80° there is no danger of the precipitation of titanium phosphate under these conditions.

The phosphate test is also very sensitive when the concentration of sulphuric acid is 005N or lower, but it is not permissible to use such low concentrations on account of the danger of precipitation of ferric or thallic phosphates. In order to determine the minimum limit of H_2SO_4 concentration which will keep these two phosphates in solution mixture of 10 c.c. 10 per cent Na_2HPO_4 and 10 c.c. H_2SO_4 solutions of different concentrations were treated with ferric and thallic salts with the results shown in Table IV.

TABLE IV

	10 c.c. 10% Na_2HPO_4 10 c.c. 0·1N H_2SO_4	10 c.c. 10% Na_2HPO_4 10 c.c. 0·5N H_2SO_4	10 c.c. 10% Na_2HPO_4 10 c.c. 1N H_2SO_4
100 mgm Fe	Yellow precipitate	White precipitate	No precipitate
100 mgm Tl	Yellow precipitate	No precipitate	No precipitate

From the above results we see that when the solution is 0·5N with respect to H_2SO_4 and contains 100 mgm either of iron or thallium, neither ferric phosphate nor thallic phosphate is precipitated If therefore the concentration of sulphuric acid is above 1N there is no danger of the precipitation of either Fe of Tl as phosphate

On making a test analysis with 100 mgm each of *thallium, iron, and titanium and 0 5 mgm zirconium*, no precipitate of zirconium phosphate was obtained even in an hour, although the quantity of zirconium was more than sufficient to give a precipitate according to results given in Table III. By making test analyses, combining only one metal in large quantity with a small quantity of zirconium it was found that it was iron which hindered the immediate precipitation of zirconium in small quantities In presence of 100 mgm. iron even 1 mgm. of zirconium is not immediately precipitated though in its absence 0·5 mgm zirconium gives a clear flocculent precipitate A precipitate is, however, obtained with 1·5 mgm zirconium even in presence of 100 mgm iron In presence of iron, the phosphate test for zirconium is rendered slightly less delicate but it is sufficiently delicate for qualitative purposes in ordinary class work

From a consideration of the tests discussed above the following procedure for the analysis of the precipitate containing the hydroxides of the four metals may be devised —

Treat the precipitate of the hydroxides with about 5N H_2SO_4 solution until it just dissolves. Then add an equal volume of 2 5N H_2SO_4, and filter the solution if it is not quite clear. In this way a solution will be obtained with a normality lying between 2 5N and 1·25N. The object should be to keep the total volume of the solution as small as possible and if the quantity of the precipitate is very large, sulphuric acid with a normality greater than

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5N should be used for the preliminary neutralization of the hydroxides

(*a*) To a small portion of the solution (about 2 c c.) add 1 c c 1N KI solution and 5 c c saturated SO_2 solution The formation of a yellow precipitate proves the presence of thallium A yellow colour alone does not indicate the presence of thallium, because when KI and H_2SO_4 solutions are mixed, a yellow colour is obtained

(*b*) To another small portion of the solution (about 2 c c.) add 5 c c. 1N KCNS solution a blood-red colour shows the presence of iron Since this is an extremely delicate test for iron if a light red colour is obtained, a blank test should be performed with the acids used in the previous procedures, to see whether these are contaminated with traces of iron If it is necessary to add ferric chloride for the separation of phosphoric acid from metals of the alkaline earth group, iron should have been tested for before the addition of ferric chloride solution.

(*c*) To the remaining portion of the solution add 5—10 c.c 3 per cent H_2O_2 solution A yellow to orange colour indicates the presence of titanium If the solution before the addition of H_2O_2 is slightly yellow owing to the presence of a large quantity of iron, dilute it with water till the colour is almost inappreciable

(*d*) To the solution which has been tested for with H_2O_2, add 5 c.c. 10 per cent Na_2 PHO_4 solution. Heat to about 70°— 80°. A white flocculent precipitate proves the presence of zirconium As very small quantities of zirconium (less than 1 mgm) are precipitated slowly, the solution should be examined again after about an hour to see if a slight precipitate has separated during this time

The procedure is practically the same when phosphoric acid is removed from the solution by means of ferric chloride in presence of sodium acetate and acetic acid, and the mixed precipitate consists of phosphates, basic acetates

or hydroxides of the four elements, but the following points should be borne in mind in this case .

(*i*) Iron should be tested for in the usual way before adding ferric chloride for elimination of phosphoric acid from the solution

(*ii*) Zirconium and titanium are not likely to be present since the phosphates of these metals are insoluble in dilute mineral acids.

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CHEMICAL EXAMINATION OF THE KERNELS OF THE FRUIT OF THEVETIA NERIFOLIA (JUSS)

BY

NARENDRANATH GHATAK, M Sc,

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Thevetia nerifolia or yellow oleandor as it is known in English and Pila-kaner in Hindustani is a plant of the natural order Apocynaceae, commonly cultivated in India as an ornamental garden shrub. The fresh bark of the young wood, of from ½—1 inch in diameter, is green, smooth and covered by a thin grey epidermis, through which the green colour is apparent; it turns black when dry. All parts of the plant yield an abundance of acrid milky juice The flowers are yellow. The fruit is globular, slightly fleshy, green, 1 to 2 inches in diameter, and contains a hard nut, light brown in colour and triangular with a deep groove along the edge corresponding to the base of the triangle; each nut contains two pale yellow, slightly winged seeds with a light brown coating.

Descourtilz, in his "Flora of the Antilles," speaks of Thevetia nerifolia as an acrid poison, of the bark as a drastic purgative, of the fruit as emetic and of an extract of the plant as a remedy for intermittent fever. He describes the case of a young negro who had eaten of the green fruit, and who was affected with chills, delirium, and other nervous symptoms, nausea and a thready pulse; he had irregular spasms, followed by extreme agitation, with singing, laughing, and weeping and then followed by a

"Thevetosin" by the present author It was optically active having a positive rotation

After removing thevetosin from the alcoholic extract it was completely freed from chloroform A lump of light yellowish brown mass was obtained It was very hygroscopic and contained a glucoside very soluble in water, glucose and other water-soluble impurities This glucoside was "thevetin," the active principle of the kernels, which on hydrolysis with mineral acids gave Warden's (loc. cit.) thevetin-blue Thevetidine, the genin of thevetin, separates readily on hydrolysis as a light brown oily liquid. It very soon gets oxidised changing colour to greenish blue, blue and finally into a black mass. It is little soluble in alcohol and almost insoluble in all organic solvents excepting pyridine, in which it is very soluble forming a brownish black solution All methods of getting thevetin in a pure form failed Ultimately recourse was taken to the study of the genin in order to throw some light on the constitution of thevetin. Thevetidine on simultaneous reduction and acetylation gave a pale yellow micro crystalline, hygroscopic powder melting at 93°C.

EXPERIMENTAL

The kernels of the nuts contained 22·95 per cent of moisture and weighed 22 6 per cent of the whole nut. The kernels contained 6 4 per cent of a thin light brown coating. The average weight of a kernel was 35 gm and it contained 7 4 per cent of moisture

In order to test the presence of enzymes, the crushed kernels were kept in water. But the presence of oil formed an emulsion which could not be separated Next time 50 gms of the crushed kernels were put in a flask with petroleum ether for several hours. It was filtered and the oil removed by distilling off the petroleum ether This was repeated several times till the kernels contained no oil. The

kernels were then put in an open dish for the petroleum ether to escape The dry powder was then put in a flask with water at the room temperature for three days Few drops of chloroform were added to stop bacterial growth. It was filtered and ethyl alcohol was added to the filtrate. A white flaky precipitate slowly settled at the bottom showing the presence of enzymes.

10 gms of the kernels were tested for the presence of alkaloids, but with negative result.

After completely burning the kernels 1 9 per cent of white residue (ash) was obtained, which contained 3'1 per cent of SiO_2. The soluble portion of the ash contained phosphate and magnesium

For complete analysis 1 5 kilograms of the kernels were crushed and exhaustively extracted with 5 litres of petroleum ether (B P 35—60°C.) in a round bottom extraction flask, till the extract no longer gave any oily residue. The total quantity of oil obtained amounted to 1030 gms. which corresponded to 68'6 per cent of the kernels A current of air was passed through the oil for about 40 minutes to drive off the petroleum ether. For further purification the oil was treated with animal charcoal, little quick lime and Fuller's earth. It was heated over water-bath and stirred for some time On filtration a very light yellow transparent non-drying oil was obtained The oil has been worked out by Bhattacharya and Ayyar (loc. cit).

The refractive index of the oil at different temperatures was determined by means of a Pulfrich refractometer :

Temp.	Observed reading	Refractive index.
10°C.	42° 44′	1 47195
29°C.	43° 15′	1'46689
30°C.	43° 45′	1'46593
40°C	44° 22′	1 46225
50°C.	44° 59′	1 45856

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10 gms of the kernels were tested for the presence of alkaloids, but with negative result.

After completely burning the kernels 1·9 per cent of white residue (ash) was obtained, which contained 3·1 per cent of SiO_2 The soluble portion of the ash contained phosphate and magnesium

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40°C ..	44° 22' ...	1·46225
50°C. ..	44° 59' .	1·45850

The kernels were freed from petroleum ether and successively extracted with alcohol till the extract gave traces of residue on evaporation The alcoholic extract was concentrated under reduced pressure when a thick brown syrupy liquid, strongly smelling of sugar, was obtained This slowly solidified to a brown mass in a vacuum desiccator On extraction with chloroform it gave 45 gms of yellowish brown solid which was completely soluble in ethyl acetate Traces of oil that was contaminated with it was removed by petroleum ether On crystallization from dilute alcohol it was obtained as fine white needles melting at 194°C It dissolved in strong sulphuric acid with the production of a yellow colour which slowly changed to pink and finally to a cherry-red. This product was thevetosin, the water insoluble glucoside. It reduced Fehling's solution readily after being hydrolysed with dilute hydrochloric or sulphuric acids. It was soluble in organic solvents excepting benzene and gave a positive rotation of $[\alpha]_D^{35} = +66\,85$ in absolute alcohol. On combusting the substance the following results were obtained .

C=64·95 per cent ; H=8·22 per cent ; and therefore O=26 83 per cent

Hydrolysis of Thevetosin—3 gms. of thevetosin was dissolved in 200 c c. of ethyl alcohol and 150 c c. of water containing 2 5 c c of HCl (d 1 16) was added. It was refluxed for about an hour. The solution was cooled and carefully neutralised with sodium carbonate. It was next concentrated under reduced pressure A semi-solid brown, sticky substance separated This was the genin—'thevetosidine.' On crystallization from alcohol and animal charcoal it was obtained in the form of a light brown liquid sticky mass which settled at the bottom. After sufficient of the substance had separated the upper liquid was removed and the product was washed several times with distilled water.

After few days it became brittle, when it was powdered and put in a vacuum desiccator It was finally obtained as a yellowish brown micro-crystalline powder melting at 83°C. Concentrated sulphuric acid produced a pink-red coloration with a green fluorescence In strong nitric acid it dissolved with a yellow coloration. Alcoholic solution of the substance did not give any precipitate or colour reaction with ferric chloride

The mother liquor after the separation of thevetidine was concentrated and finally evaporated to dryness in reduced pressure The residue was dried over H_2SO_4 in vacuum desiccator and was extracted with dry acetone. The extract was evaporated to dryness This was the sugar of hydrolysis It reduced Fehling's solution readily. An attempt was made to crystallize it from ethyl acetate but instead of getting a better stuff, a brown sticky substance was obtained. The quantity being small, the sugar could not be identified.

Thevetin.—The product left after the separation of thevetosin by chloroform extraction contained another glucoside, thevetin, which was very hygroscopic It contained some free glucose, albumenous product and other water-soluble impurities. All methods of separating the glucoside in a pure form having failed, recourse was taken to the study of the gluco-genin obtained on hydrolysing thevetin.

Hydrolysis of Thevetin —The same experimental procedure was followed in the hydrolysis of thevetin as in the previous one. Hydrolysis was effected very soon in this case. Thevetidine, the gluco-genin, first separated as a brown semi-solid mass which slowly got oxidised in the presence of air changing colour to green, blue and finally into a black mass. Thevetidine was very little soluble in alcohol and was almost insoluble in all organic solvents with the exception of pyridine in which it was considerably soluble forming a brownish-black solution. A little of the

In order to explain the mechanism[1] of these induced oxidations in the presence of ferrous and cerous salts, the formation of higher oxides like FeO_2 (Manchot[2]) and Ce_2O_5 (Job[3]) has been assumed, and these higher oxides oxidise the difficultly oxidisable substances like the food materials

Our experimental results[4] on the oxidation of sodium formate by air in presence of ferrous and cerous hydroxides lend support to the hypothesis of the intermediate formation of the higher oxides

From our experimental results[5] on the induced oxidation of glucose by air in presence of ferrous and cerous hydroxides, it will be seen that the induction factor, *i e*, the ratio of the amount of oxygen taken up by glucose to the amount of oxygen taken up by inductor is as high as 8 or 9 Similar results are also obtained with other reactions. Spoehr also obtained a value as high as 15 for the induction factor It, therefore, appears that the oxidised form of the inductor, *viz*, Fe_2O_3 or CeO_2, etc, is also capable of oxidising the acceptor thereby regenerating the original inductor. Hence these induced reactions appear to be partly catalytic in nature but inasmuch as the rate at which the original inductor is regenerated is small as compared with the rate of its oxidation, these reactions belong more to the induced type rather than the catalytic one

These higher values of the induction factors can be satisfactorily explained from the point of view of the generation of ions in the primary exothermal reaction Thus, for example, a small amount of an inductor is oxidised; some ions will be generated in this exothermal reaction and

[1] Jour Phy Chem, *35*, 2043 (1931)
[2] Ann, *314*, 177 (1899), *325*, 93 (1902), *460*, 179 (1927)
[3] Job, Ann Chem Phys, (7) 20, 207 (1900)
[4] Palit and Dhar, Jour Phy Chem, *34*, 711 (1930)
[5] Palit and Dhar Jour Phy Chem, *29*, 799 (1925), *30*, 959 (1926).

the ions will activate some molecules of the acceptor or the actor or both These then will react. This reaction being exothermal will in its turn give rise to more ions which will activate some more molecules of the reactants and so on Thus the oxidation of a small quantity of the inductor brings about the oxidation of a large amount of the acceptor, that is, the slow oxidation of the reducing agents (inductors) set up the oxidation of carbohydrates, fats, proteins and other food materials

It is well known that the edible substances like carbohydrates, fats and proteins are very readily oxidised in the body, whereas they are oxidised with difficulty by ordinary laboratory reagents We have carried on our experiments[1] and have been successfully able to induce in the laboratory the oxidation of edible substances like glucose, starch, milk, butter, egg-white, egg-yellow and also the oxidation of other substances like cholesterol, lecithin, glycerol, etc , at the ordinary temperature by passing a slow stream of air in presence of inductors like sodium sulphite, ferrous hydroxide, cerous hydroxide, etc.

It has also been shown[2] that not only fats but carbohydrates and nitrogenous substances are oxidised by hydrogen peroxide and a ferric salt at 37°, volatile aldehydric or ketonic compounds being formed. We[3] have conclusively proved in a systematic manner that fats, carbohydrates and nitrogenous and other organic substances can be completely oxidised into their main end products, carbon dioxide and water, by air with the help of an inductor, ferrous or cerous hydroxide or in presence of sunlight at the ordinary temperatures and we have thus been able to imitate successfully the physiological process of oxidation on which animal

[1] Jour. Phy. Chem., *31*, 711 (1927).
[2] Jour. Ind. Chem. Soc., *6*, 617 (1929).
[3] Jour. Phy. Chem., *31*, 711 (1927).

good reducing agents and are readily oxidised by atmospheric oxygen and the oxidation of these substances induces the oxidation of sugar in the body We have now been able to substantiate this view by our new sets of oxidation experiments on insulin and glucose For these experiments a definite volume of air freed for carbon dioxide was passed through an aqueous solution of insulin (B D H) kept at 25° and the amount of carbon dioxide obtained by oxidation of insulin was absorbed by standard barium hydroxide solution and estimated as usual When glucose is added to the insulin solution and the same volume of air is passed through the mixture glucose is slowly oxidised and this can be shown by estimation of glucose by Fehling's solution, which, however, cannot be reduced by insulin In this experiment with insulin and glucose, the oxidation of insulin which is readily oxidised by air at ordinary temperature leads to the oxidation of glucose thus corroborating our previous statements

In several publications,[1] we have emphasised the importance of induced oxidations in understanding the phenomenon of animal metabolism It has been stated that the readily oxidisable substances like glutathionone and other substances present in muscle and in other parts of the body, are first oxidised by the inhaled oxygen and these oxidations induce the oxidation of food materials. Insulin and other internal secretions also appear to be readily oxidised in the body and these lead to the oxidation of carbohydrates, fats and proteins. It is now well-known that in the treatment of acute diabetes, repeated doses of insulin have to be injected in order to get satisfactory results. Our experiments on the oxidation of insulin by air show *that it is used up by the oxidation in the body* and thus repeated doses are necessary Moreover, the

[1] Jour. Phy Chem , 35, 2043 (1931)

oxidation of insulin leads to the oxidation of glucose in the body and thus explains the decrease of glucose in the diabetic blood and urine on injection of insulin

(4) Animal life is assumed to depend essentially on the catalytic activity of the enzymes and iron in the animal body It is likely that in the animal body, there exist readily oxidisable substances such as enzymes containing traces of iron in complex colloidal condition and the oxidation of these substances induces the oxidation of food materials

(5) In the animal body, the iron in the blood accelerates catalytically the oxidation of food stuff by the peroxide formed in the body from the inhaled oxygen When there is a deficiency of iron in the blood, the animal becomes anæmic At this stage any iron salt preferably of colloidal nature taken in the body, will supply the natural deficiency and the necessary amount of oxidation will take place.

(6) We also suggest that fever is an auto-catalytic reaction. The oxidation of substances like starch, sugar, proteins, fats, etc , by oxygen in the animal body is believed to be catalytically accelerated by the parasites or secretions of bacteria Hence the amount of heat generated in the animal body for unit time is increased and the phenomenon of fever is observed. Moreover, like all other chemical changes, the amount of oxidation in the animal body for unit time is also increased by the incipient rise of temperature.

(7) From our quantitative experiments[1] on the oxidation of carbohydrates, glycerol, fats and proteins by air in presence of freshly precipitated ferrous and cerous hydroxides and sodium sulphite as inductors, we have shown that the amount of carbon dioxide obtained in these slow oxidations is practically the same as is expected from the

[1] Palit and Dhar, Jour. Phy. Chem , *34*, 711 (1930); Zeit. anorg allgem Chem , *191*, 150 (1930)

point of view that the carbohydrates, glycerol, fats and nitrogenous substances are completely oxidised into carbon dioxide and water by passing air at the ordinary temperature. Similarly, Spoehr has obtained considerable amounts of carbon dioxide from the induced oxidation of carbohydrates by air in presence of sodium, ferrous and ferric pyrophosphates We are of opinion that these results are of importance because these oxidations are of the same type as those taking place in the animal body. Hence we emphasise that in normal health, the food materials taken in the body are completely oxidised into carbon dioxide and water without the formation of intermediate compounds, just as food materials are completely oxidised to carbon dioxide and water when air is passed through their solutions or suspensions in presence of inductors. Intermediate compounds are only formed in the diseased condition of the animal body

(8) Voit stated " that the metabolism in the body was not proportional to the combustibility of the substances outside the body, but that proteins which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat which readily burns outside is the most difficultly combustible in the body." This conclusion was arrived at by Voit from actual feeding experiments on animals We have obtained quantitative and comparative results[1] on the velocity of oxidation of fats, proteins and carbohydrates by air and thus tried to establish whether fats or carbohydrates are oxidised more readily in the system Our results show that the order in which they are oxidised in presence of cerous hydroxide are as follows.

Egg-white > egg-yellow > starch > glucose > butter.

In presence of cerous hydroxide, the induced oxidation of fats, nitrogenous substances and carbohydrates follows the same order as stated by Voit

[1] Jour. Phy Chem , *34*, 711 (1930)

(9) The experimental results[1] show that carbohydrates, proteins, fats and other substances are oxidised in presence of inductors in neutral and alkaline solutions, and the greater the amount of alkali, the greater is the amount of oxidation. Hence we are of opinion that alkaline treatment should prove efficacious in gout, diabetes, beriberi, rickets and other metabolism diseases, because in presence of even sodium bicarbonate, the amount of oxidation of fats, carbohydrates and nitrogenous substances is greatly increased.

Hence all these results on slow and induced oxidation of fats, nitrogenous substances and carbohydrates occurring either singly or in mixtures by air at ordinary temperature are important, because those oxidations are of the same type as those taking place in the animal body.

Photo-Chemical Oxidations and Physiological Experiments

Aqueous solutions or suspensions of the following substances have been oxidised by passing air in presence of sunlight[2] :

Arabinose, cane sugar, galactose, glucose, lactose, laevulose, maltose, starch, glycogen, urea, glycine, α-alanine, hippuric acid, sodium urate, potassium oxalate, sodium formate, sodium tartrate, potassium stearate, potassium oleate, potassium palmitate, lecithin, glycerol, cholesterol, butter, egg-white, egg-yellow and milk. Zinc oxide, uranium nitrate and ferric nitrate act each as a photo-sensitiser in the oxidation of the above substances and the amount of oxidation of these substances is greater than that in their absence.

[1] Palit and Dhar, Jour Phy. Chem., 29, 709 (1925) ; 30, 939 (1926).

[2] Palit and Dhar, Jour Phy. Chem., 32, 1983 (1928), 34, 593 (1930) ; and Zeit. anorg allgem Chem., 191, 120 (1930).

1 Our results[1] also show that the amount of oxidation increases with (i) the intensity of light, (ii) the amount of light falling in the solutions, and (iii) the time of exposure.

2 Dilute solutions of lactic acid, oxalic acid, tartaric acid and citric acid are appreciably oxidised by air in presence of sunlight and the order in which they are oxidised is

Oxalic > lactic > tartaric > citric

3 In order to find out whether in presence of sunlight the carbohydrates, fats, and nitrogenous substances are oxidised completely to carbon dioxide or other intermediate products are formed, we have estimated the amount of carbon dioxide obtained in these oxidations in potash bulbs. The amount of oxidation of these substances was also, in all cases, estimated by direct analysis The experimental results[2] show that the amount of oxidation determined from carbon dioxide obtained is practically the same as the oxidation found out from the direct analysis of the carbohydrates, fats and nitrogenous substances remaining unoxidised Hence in presence of sunlight, different carbohydrates, fats and nitrogenous substances can be completely oxidised by air at the ordinary temperature into their main end products, carbon dioxide and water. No intermediate compounds are formed in these photo-chemical oxidations. We have thus been able to imitate successfully the physiological processes of oxidations on which animal life depends

4. Voit in his necrology of Pettenkofer writes : "That the metabolism in the body was not proportional to the combustibility of the substances outside the body, but proteins which burns with difficulty outside metabolises with the greatest ease, then carbohydrates, while fat

[1] Palit and Dhar, Jour Phy Chem , *32*, 1263 (1928), *34*, 903 (1930) and Zeit anorg allgem Chem , *191*, 150 (1930)

[2] Ibid

which readily burns outside is the most difficultly combustible in the body." We have tried to imitate the metabolism taking place in the animal body and have made comparative experiments[1] on the oxidation of butter, egg-white, egg-yellow, starch, glycogen, and glucose by passing air at the ordinary temperature in presence of sunlight The following results have been obtained

Egg-yellow	..	60 9	per cent oxidised
Egg-white		31 25	,, ,,
Starch		39 2	,, ,,
Butter	.	31 8	,, ,,
Glucose		13 6	,, ,,

It appears, therefore, that egg-yellow is the most easily oxidisable substance in presence of light, then come starch, egg-white, and butter, while glucose is the least oxidisable Hence eggs which metabolise readily in the animal body are also easily oxidised by air at the ordinary temperature in presence of sunlight.

5 We have investigated whether the Einstein Law of Photochemical Equivalence is applicable to the photochemical oxidation of carbohydrates, fats and nitrogenous substances in sunlight. The amount of energy absorbed by solutions of carbohydrates, fats and nitrogenous substances was measured with the help of Boys' radiomicrometer It is interesting to note that the Einstein Law of Photochemical Equivalence is applicable to the photo-chemical oxidation of glucose, lactose and α-alanine by air The law, however, is not applicable to the photochemical oxidation of glycine by air where about seven molecules react per quantum of light absorbed These results show that practically colourless one per cent aqueous solutions of glucose, lactose, glycine and alanine can absorb light from the sunshine falling on the solutions.

[1] Jour. Phy Chem., *34*, 993 (1930).

This absorption of energy leads to the activation of the molecules and their consequent chemical reaction with oxygen in presence of light When these solutions are mixed with ferric or uranium nitrate, the absorption of radiation is considerably increased and the amount of oxidation is also increased.

6 In one of our previous publications,[1] we have shown that appreciable amounts of the compounds of the peroxido type are formed when air is passed through aqueous suspensions of cholesterol, olive oil, butter and many other substances like cocoanut oil, castor oil, linseed oil, mustard oil, etc It has been also observed that olive oil can be retained in that activated or excited state for a sufficient length of time if kept in the dark but this phenomenon was not observed in a marked degree in the case of cholestorol, as it was found to have lost its active or excited state in the course of a few days Moreover, appreciable amounts of glucose have been oxidised by mixing the solution of glucose with the exposed cholesterol, olive oil, butter, and other oils respectively, containing the peroxide compounds Hence it is believed that the anti-rachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxido, which help the oxidation of food materials in the animal body. Substances can acquire anti-rachitic properties when exposed to light only in presence of air and light.

In the light of the observations made we can safely say that when the food materials are exposed to sunlight in presence of air, they take up oxygen forming some peroxide type of compound which can oxidise other food materials when mixed with them. Consequently the addition of the exposed substances to ordinary food stuff facili-

[1] Jour Phy Chem., *34*, 737 (1930), *34*, 993 (1930), Ind. Jour Med. Research, *17*, 420 (1929)

tates the proper ingestion of food materials and produce efficacious results

7. Sunlight and artificial lights have been used with great success in the treatment of tuberculosis, pernicious anæmia, rickets, etc. In some previous publications[1] we have emphasised the importance of sunlight in the treatment of deficiency diseases and we have observed that rickets, osteomalcia, beri-beri, pellagra, etc., would have been more common in poor tropical countries like India and China, had not the compensating agent—sunlight—been present. This conclusion has been corroborated by our experiments on the metabolism of animals

8. Having investigated the above facts on the efficacy of exposed oils in oxidising other food materials, we have carried on experiments[2] on the metabolism of pigeons and rats using these exposed and unexposed oils. Incidentally we have also investigated the influence of sunlight and small quantities of colloidal iron preparations, juice of several green leafy vegetables, tomato, etc., in the metabolism of pigeons and rats For this, different lots of pigeons and rats were fed on polished Rangoon rice which is believed to be entirely devoid of vitamins for about a month. One lot had plenty of sunlight, whilst the other had very little of it. The lot which had sunshine did not show any sign of polyneuritis whilst the other lot not having sunshine developed stomachic troubles first and then acute form of polyneuritis, paralysis and their eyes were highly affected. All the affected pigeons were separated from the rest and kept in sunlight and fed artificially with substances rich in vitamins and containing iron in small

[1] Jour. Phy. Chem., *32*, 1203 (1928); *33*, 1807 (1929) and Chemie der zelle und Gewebe., *12*, 217, 225, 317 (1925); *13*, 209 (1926).

[2] Jour Phy Chem., *32*, 737 (1930); and Ind Jour. Med. Research, *17*, 430 (1929).

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[1] Jour. Phy. Chem., 32 1281 (1928), 33, 1907 (1929) and Comm der soils and foodst., 13 217, 223, 317 (1928), 14, 331 (1929).

[2] Jour Phy. Chem., 32. 771 (1928), and Ind Jour Med Research, 17, 1 (1929).

SUMMARY

(1) An explanation for the mechanism of induced oxidation has been suggested

(2) Carbohydrates, fats, proteins, food materials, and other organic substances have in presence of inductors been oxidised

(3) The oxidation of fats is retarded by carbohydrates or less powerfully by proteins and to a greater extent by a mixture of proteins and carbohydrates Also the oxidation of proteins is markedly retarded by fats and carbohydrates

(4) The view that diabetes is due to insufficient oxidation of glucose and fats in the body, has been corroborated by our experimental evidence on the oxidation of insulin, which goes to prove that the oxidation of insulin leads to oxidation of glucose in the body Thus explains the decrease of glucose in, and disappearance of acetone bodies from, the diabetic blood and urine on injection of insulin.

(5) Iron in the blood accelerates catalytically the oxidation of food materials The iron preferably of colloidal nature, when taken into the system, will supply the natural deficiency and the necessary amount of oxidation will take place, thus showing the efficiency of the iron preparations in deficiency and metabolism diseases An explanation that fever is an autocatalytic reaction has also been suggested

(6) Experimental results on the estimation of carbon dioxide prove that carbohydrates, fats, proteins and other organic substances are oxidised by air at the ordinary temperature in presence of inductor chiefly to carbon dioxide and not to any intermediate products

(7) Comparative experiments on the induced oxidation of fats, carbohydrates, and proteins show that in presence of inductor, the order of oxidation is the same as that obtained by Voit, the eminent physiologist.

(8) An explanation of the internal use of alkali and alkaline carbonates has been suggested based on the increased oxidation of food materials by air in presence of alkali The alkaline treatment

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(8) An explanation of the internal use of alkali and alkaline carbonates has been suggested based on the increased oxidation of food materials by air in presence of alkali. The alkaline treatment

should prove efficacious in gout, diabetes, beri-beri, rickets and other metabolism diseases

(9) Aqueous solutions or suspensions of the following substances have been oxidised by passing air in presence of sunlight — arabinose, cane-sugar, galactose, glucose, lactose, laevulose, maltose, starch, glycogen, urea, glycerine, α-alanine, hippuric acid, sodium urate, potassium stearate, potassium oleate, potassium palmitate, potassium oxalate, sodium formate, sodium tartrate, lecithin, cholesterol, butter, milk, egg-white, egg-yellow and dilute solutions of citric, tartaric and lactic acids Zinc oxide, uranium nitrate and ferric nitrate act as a powerful photosensitiser in the above oxidations and in their presence the amount of oxidation in each case is greater than in their absence. Our experimental results show that the amount of oxidation increases with (*a*) the intensity of light, (*b*) the amount of light falling on the solutions, and (*c*) the time of exposure.

(10) Experimental results on the estimation of carbon dioxide prove that carbohydrates, fats, proteins, food materials, cholesterol, lecithin, etc, are oxidised by air in presence of sunlight chiefly to carbon dioxide and not to any intermediate product

(11) Comparative experiments show that order in which the food materials are oxidised in presence of sunlight is as follows —

egg-yellow > starch > egg-white > butter > glucose.

(12) The Einstein Law of Photochemical Equivalence is approximately applicable to the photochemical oxidations of glucose, lactose and alanine by air.

(13) Experimental results show that appreciable amounts of the compounds of the peroxide type are formed when air is passed through aqueous suspensions of cholesterol, butter, olive, cocoanut, mustard, castor, and linseed oils and some carbohydrates. These peroxides have been estimated by the amount of iodine liberated by them from an acid solution of potassium iodide Moreover, appreciable amounts of glucose have been oxidised by mixing the solution of glucose with the exposed substances containing the peroxide compound. Hence it is believed that the anti-rachitic and beneficial properties of substances not containing the necessary vitamins are due to the presence of peroxides which help the oxidation of food materials in the animal body

(14) From the experiments on metabolism of animals, we have proved that sunlight is the best preventive for diseases like

polyneuritis, beri-beri, rickets, etc. Olive oil, exposed to sunlight and air, comes on close second, whereas iron and unexposed oils are harmful to animals The natural food with plenty of sunlight seems to be the best kind of diet for the maintenance of health. In tropical countries many deficiency diseases are avoided due to sunlight Hence sunlight and other kinds of artificial lights prove efficacious in the treatment of diseases specially of metabolic origin.

(15) These results (induced and photochemical oxidations) are very important, because these oxidations are of the same type as those taking place in the animal body. The experiments in this investigation are in reality imitations of Nature's process of oxidation of food materials in the animal body.

SECTION III

BOTANY

THE COMPARATIVE VALUES OF VARIOUS FRESH FRUIT JUICE MEDIA IN RELATION TO THE GROWTH OF CERTAIN DEUTEROMYCETES

BY

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INTRODUCTION

The study of the Deuteromycetes under artificial culture has led to the recognition of the fact that most of the organisms are variable In these variations may take place when subjected to a number of different environmental conditions, but a change of the substratum often markedly affects their growth. Investigations on the effect of different media on the growth of such fungi have resulted in the discovery of several useful media many of which are used for the production or intensification of some particular character of the organism. Thus a starchy medium such as Rice Agar is best suited for the study of colour production by a fungus and Richard's agar has proved to be very favourable for saltations

Fruits are specially suitable for the preparation of media because of the valuable nutritive substances they contain and the ease with which their juice can be extracted. That is why a large number of fruit juice media are employed in the Western countries for the cultivation and study of the fungi. In this country it had been the practice to prepare media from those fruits only as are used in

the foreign countries, such as Prune juice agar, apple agar, etc., because their effects are known. But though a large number of fruits grow every year in India there has almost been no attempt to ascertain the relative effects of the media prepared from their juices on the growth of fungi. Moreover, as has been pointed out before, useful results are always to be obtained from the study of the suitability of so many widely varying media. According to Brown (5) the problem why one organism grows well in one medium and not on another is of "greatest interest in pathology, as its solution would form a vantage ground for the study of immunity in so far as the latter is based on nutritional factors."

The present paper is an attempt to investigate the relative values of the juices of certain Indian fruits, as media, for the cultural study of four Deuteromycetes.

Materials and Method.—At the beginning the four fungi were freed from bacteria by the method of Brown (4) and single spore cultures were prepared by the triple dilution and poured plate method. These single spore cultures were kept in plugged test tubes and were the source of all subsequent inoculations. For observations petridish cultures were made in triplicate and no character (saltants excepted) has been presented here that did not appear in all of the three plates.

Preparation of the Media.—1. Brown's synthetic medium with starch.

Asparagin	2 gms.
Magnesium sulphate	75 gm.
Glucose	2 gms.
Potassium phosphate	1·25 ,,
Starch	10 ,,
Agar	18 ,,

Distilled water to make up—1000 c.c.

Asparagin and Magnesium sulphate were dissolved separately in boiling water, the volume measured and added to the rest when cool

2. Red Mulberry Agar (*Morus indica*).—Only the very ripe deep purplish fruits were selected. They were thoroughly washed and the juice was prepared by squeezing through muslin The seeds, which were rather hard and so did not get crushed, were discarded. The juice tasted sweet but acidic.

Undiluted juice—100 c c. Agar—18 gms
Distilled water to make up—1000 c.c.

3. Green Mulberry Agar (*Morus alba*).—Only the very ripe fruits were selected and the juice prepared as in the former case. Taste—very sweet

Undiluted juice—100 c c. Agar—18 gms.
Distilled water to make up—1000 c c.

4. Water-melon Agar (*Citrullus vulgaris*).—The skin and the seeds were discarded and the juice was pressed out of the pinkish tissue through muslin.

Undiluted juice—100 c c. Agar—18 gms
Distilled water to make up—1000 c c.

5 Kakri Agar (*Cucumis utilissimus*).—The fruits were cut into small pieces and without removing the skin or the seeds were crushed thoroughly in a mortar The juice was extracted out of the pulp through muslin. Taste of the juice—flat.

Undiluted juice—100 c c. Agar—18 gms.
Distilled water to make up—1000 c.c.

6 Kharbuja Agar (*Cucumis melo*).—The skin and the seeds were discarded. The fleshy portion was pounded in a mortar and pressed through muslin. Taste—sweetish.

Undiluted juice—100 c.c Agar—18 gms.
Distilled water to make up—1000 c c.

7 Phalsa Agar (*Grewia asiatica*) Only the ripe deep purplish fruits were chosen, washed, and pressed through muslin leaving out the seeds and the fibrous parts Taste—acidic.

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c

8. Bel Agar (*Aegle Marmelos*)—The fleshy portion adhering to the hard coat and the central part with mucilage and seeds were rubbed against a stretched muslin A semi-liquid extract came out leaving the seeds and the fibrous parts. Taste—flat and mucilaginous.

Undiluted extract—50 gms. Agar—18 gms.
Distilled water to make up—1000 c.c.

9. Mango Agar (*Mangifera indica*)—The "Sindurı" variety from Madras was employed being the only kind obtainable at the time. The skin was peeled off, the fleshy portion was cut to slices and pressed through muslin. Taste—sweetish

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

10. Lichi Agar (*Gnaphalium lichi*)—Only the sweetest ones were selected. The skin and the seeds were discarded and the juice was extracted by pressing the fleshy portion through muslin. Taste—sweet

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

11. Pomegranate Agar (*Punica granatum*)—The juice was pressed out of the seeds through muslin. Taste—sweetish

Undiluted juice—100 c.c. Agar—18 gms.
Distilled water to make up—1000 c.c.

The pH-value, colour of the media, etc., are tabulated below—

Media	Symbol used *	pH-value	Colour of the juice	Colour of the media
Brown's starch	Br	7·0		Pale-olive buff.
Red Mulberry agar.	Mr	4·2	Corinthian purple	Walnut brown
Green Mulberry agar	Mg.	7·0	Yellowish white	Deep-olive buff
Water-melon agar	Wm	6·2	Deep flesh pink	Pale Cartridge buff
Kakri agar.	Ka	7·2	Light green	Pale-olive buff.
Kharbuja agar	Kh	5·6	Pinkish buff	Pale-olive buff.
Phalsa agar.	Ph	3·6	Bright Spinel Red	Pale-olive buff
Bel agar	Be	7·0	Buff yellow	Deep Apricot orange
Mango agar.	Ma	4·8	Primuline yellow	Ivory yellow
Lichi agar	Li	4·5	Pale milk white	Pale-olive buff
Pomegranate agar	Po	5·2	Pale cream	Dirty cream white

To prevent, as much as possible, the decomposition of the compounds present in the fruit juices the media were sterilized by the method of fractional sterilization, which as recommended by Harsberger (8) was done for 20 minutes at 100°C on each of the three consecutive days. In the case of Phalsa agar and Red Mulberry agar the juice and the agar had to be sterilized separately and mixed just before filling up the plates.

For the present study only those fruits were employed as could be obtained fresh from the local market at the time. Brown's starch-synthetic medium was chosen as the standard for comparison. The pH-value determinations were made by the colorimetric method. It is very difficult, if not impossible, to get correct values with this method especially if the solutions are coloured. So the results put forward are to be considered not as exact figures but only as closely approximate values. Colour identifications were made as far as possible with the help of Ridgway's (14) book.

* For convenience the media have been referred to by these symbols.

Observations on the cultures on the first six media which were inoculated at the same time to ensure identical conditions, were made at a temperature varying between 89° to 91 5° F The cultures on the rest five media, which though inoculated later were also done simultaneously, were grown at a temperature of 93° to 95° F.

Fungi used—The four fungi used in this work were growing saprophytically on various organic debris. Cultures were sent to Dr Wollenweber to whom the author is indebted for the identification of the species.

The general characters of the fungi as found on the various media are described below

Fungus No 6—*Fusarium incarnatum* (Rob) Sacc= *Fusarium semitectum* Berk et Rav variety *Majus* Wr.

Mycelium pale pink, hyaline to minutely vacuolate, septate, 3 5—5 2μ thick Spores-hyaline, slightly curved, ends gradually attenuated, apedicillate, 0 to 3 septate. Septation mode 1 Range of size 7 8 to 25 9 by 3 5 to 5 2 μ. (Plate III, 1)

Fungus No 7—*Macrosporium* sp

Mycelium deep mouse grey, slightly vacuolate, septate, 3 5—6 0 μ thick. Spores dark colored, stalked, 0 to many septate (about six), muriform, older ones rough-walled. All of the cells and even the stalk may germinate. Range of size 10 4—62 1 by 6 9—16·4 μ. (Plate III, 2 and 7)

Fungus No. 9—*Acrothecium* sp

Mycelium dark mouse grey, slightly vacuolate, septate, 3 5—5·2 μ thick, spores dark colored, the two end cells less dark than the inner ones. Spores pearshaped, elongated or bent, the amount of curvature varies. In three septate spores one of the inner cells exhibits a prominent bulging. Olive-brown to blackish grey in colour. 0 to 3 septate. Septation mode 3 but on some media 1. Germination takes place by the hyaline end cells (Plate III, 5 and 6) Range of size 6 9—25 9 by 5 2—10 4 μ.

Fungus No. 10—*Spicaria* sp.

Mycelium Pinkish white, hyaline to mostly vacuolate septate 3·5—5·2 μ thick Spores hyaline to vacuolate, oval to ellipsoidal, a septate Range of size 5·2—22·4 by 2·6—6·0 μ. (Plate III, 3)

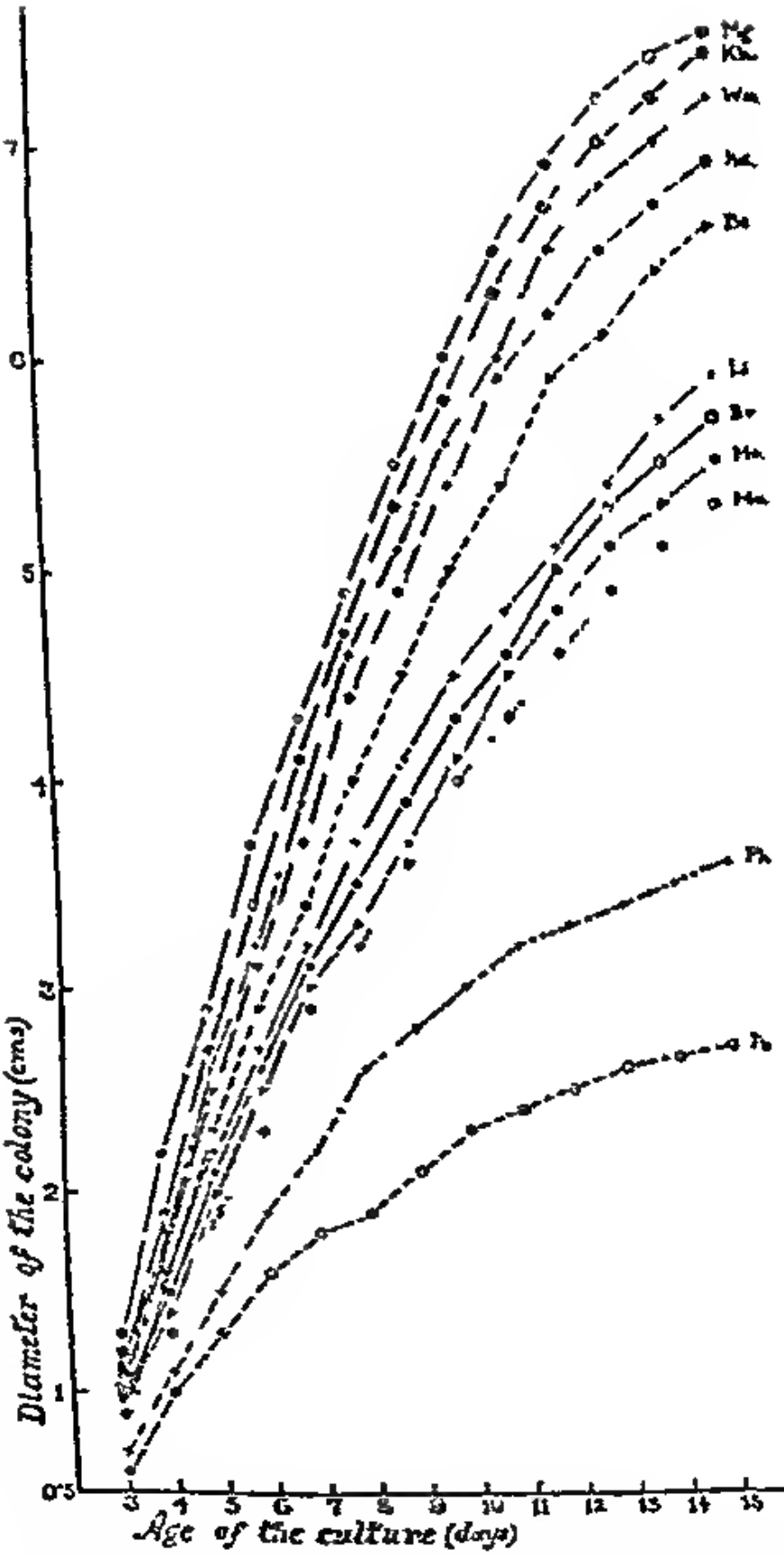
OBSERVATIONS

(A)—Macroscopic Characters

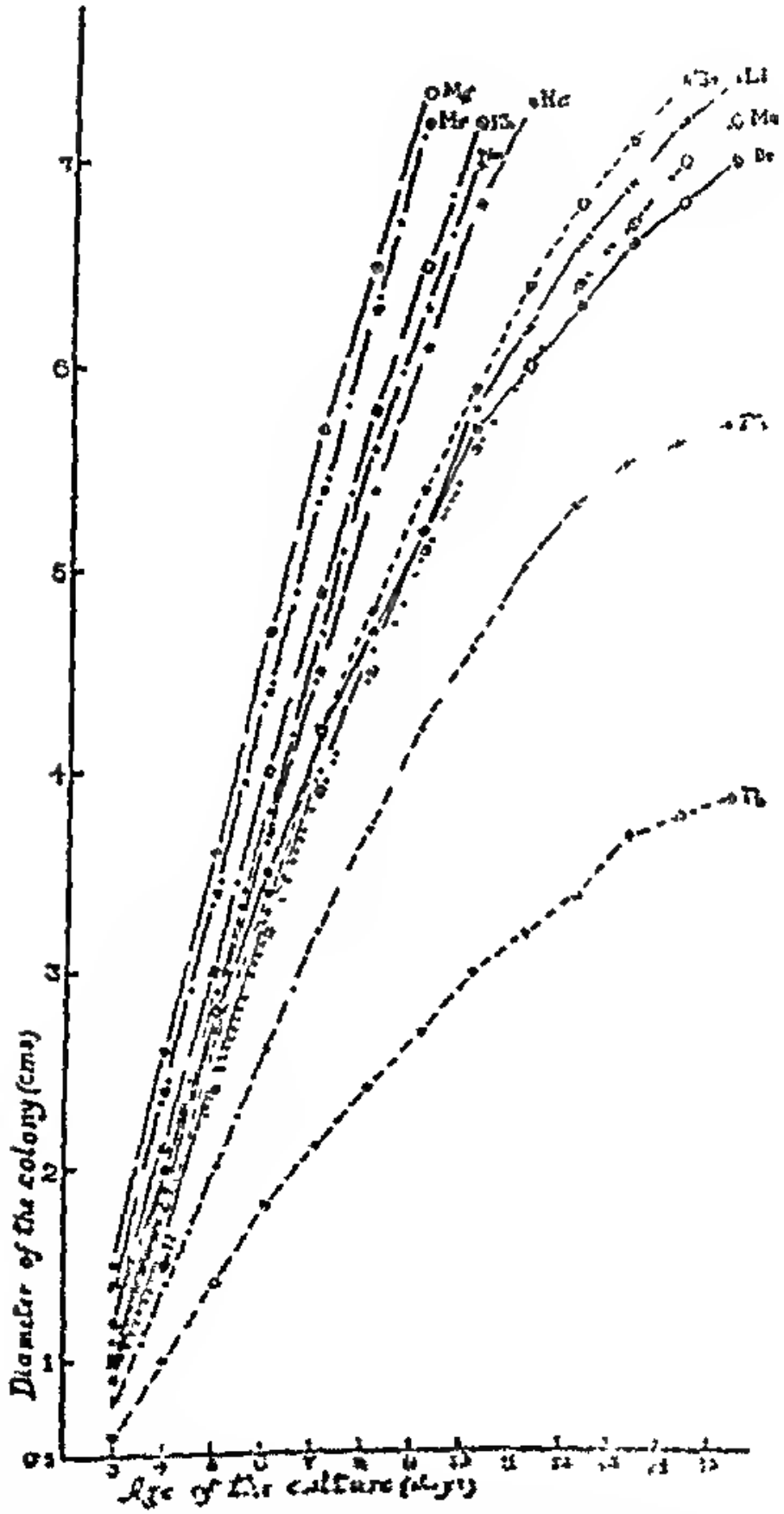
1 *Linear Growth rate*—From the graph in the Text Fig. 1 it is found that *F incarnatum* (No. 6) shows the greatest rate of diameter increase on Green Mulberry agar Its rate of growth on Kharbuja agar is also nearly as great and in fact the average rate of radial advance in both of them is the same (see Text Fig 5). On Phalsa agar the rate of spread is remarkably slow and on Pomegranate agar it is slowest—the culture not reaching more than 2·7 cms in diameter even after fifteen days' growth. All the media arranged in a series showing a descending order of growth rate are Mg, Kh, Wm, Ka, Be, Li, Br. Mr, Ma, Ph, Po.

The linear rate of spread of the fungus on all the media gradually falls off as the colony grows and thus gradual staling is shown.

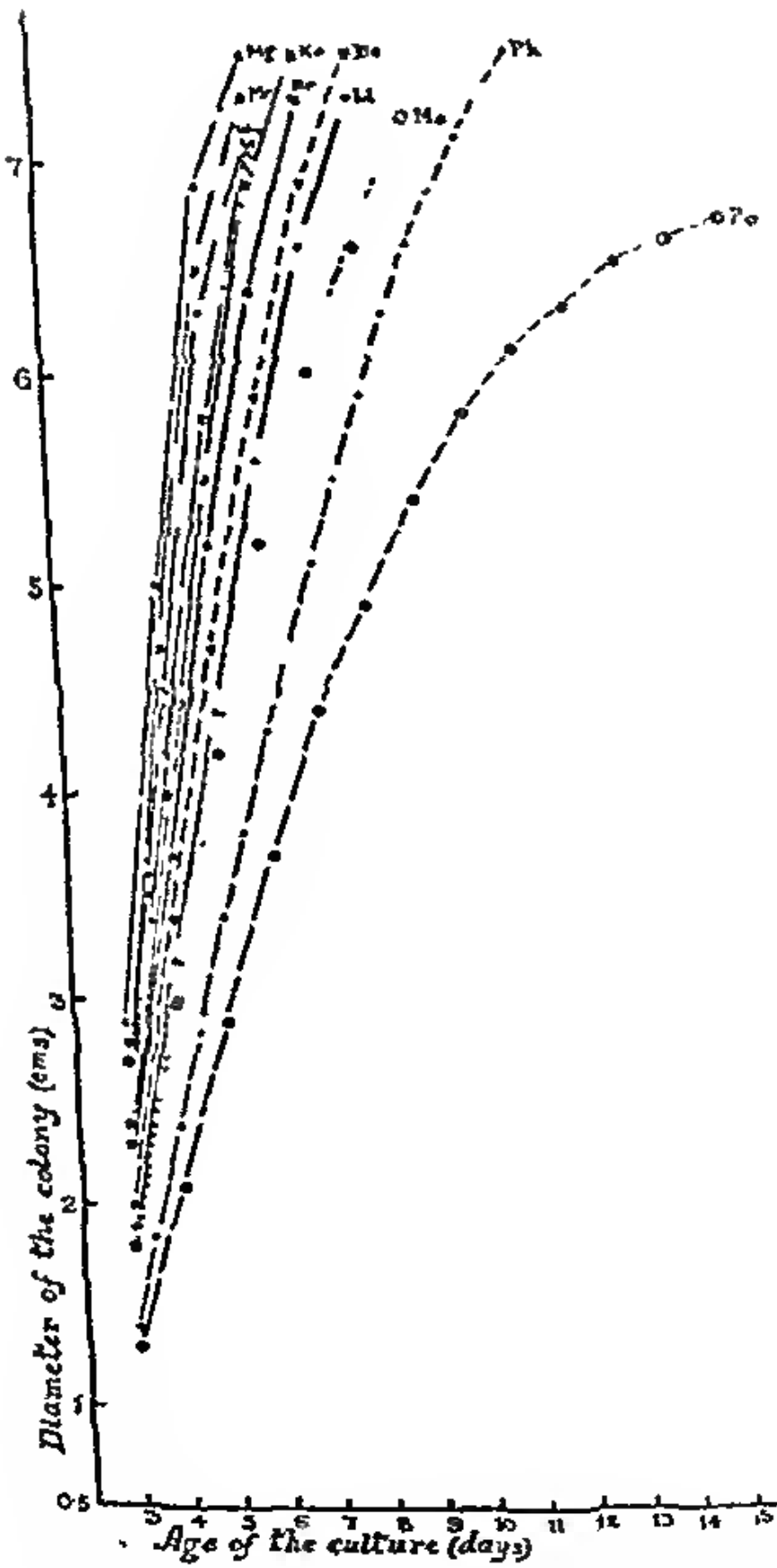
From the graph in Text Fig. No. 2 which shows the growth rate of *Macrosporium* (No. 7) it is seen that for this fungus also the most rapid growth is obtained on Green Mulberry agar and the next rapid growth is found on Red Mulberry agar. The colony on Brown's starch is staling so that though its graph begins at a higher point than many of the media, it later comes down to a lower level. On Pomegranate agar the rate of growth is slowest. The series showing a descending order of growth rate in this fungus is—Mg, Mr, Kh, Wm, Ka, Be, Li, Ma, Br, Ph, Po. The chart for the average rate of radial advance (Text Fig. 5) also shows the same series.



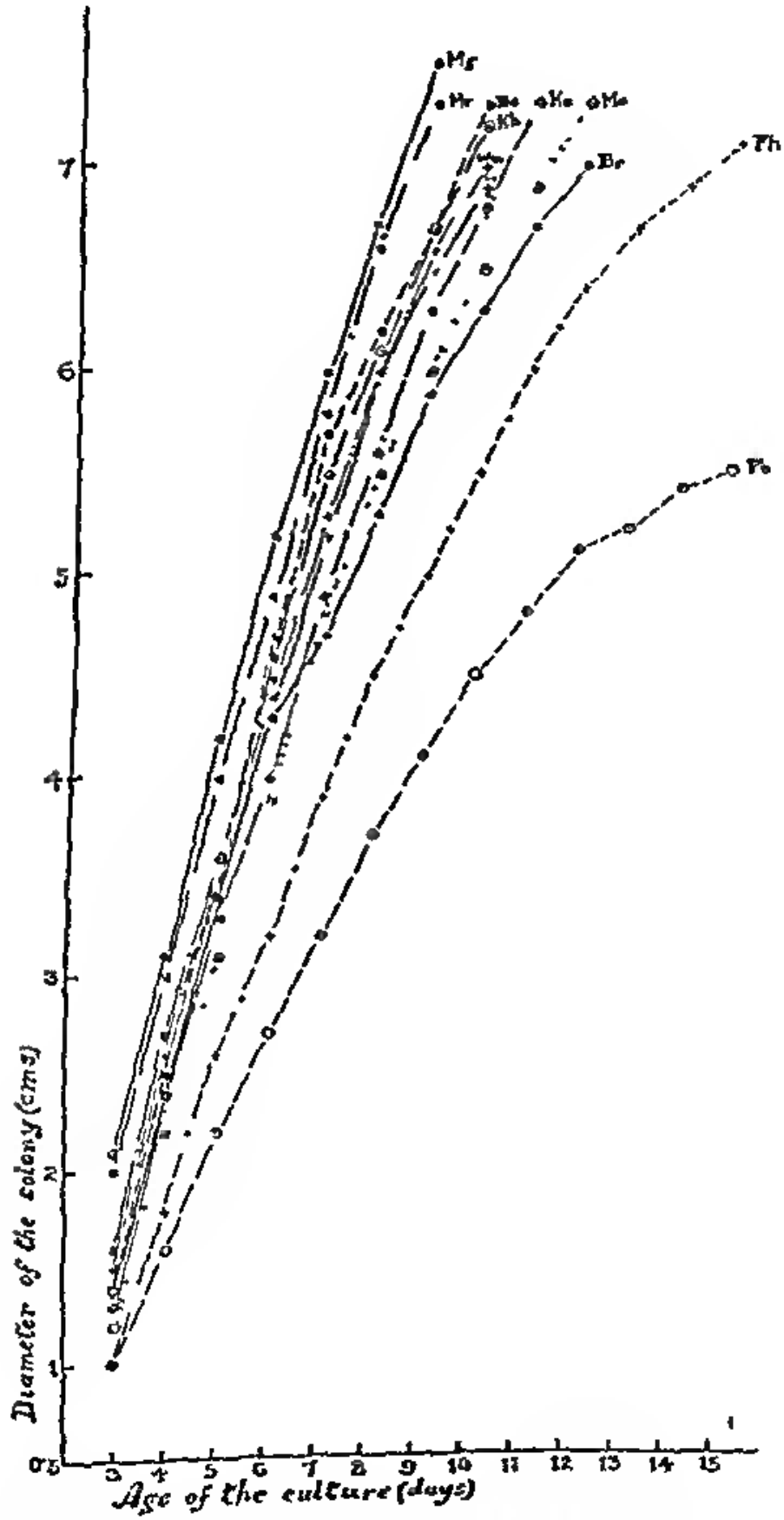
Text-Fig 1 **Graph showing the rate of linear growth of *Fusarium incarnatum* on the various media.**



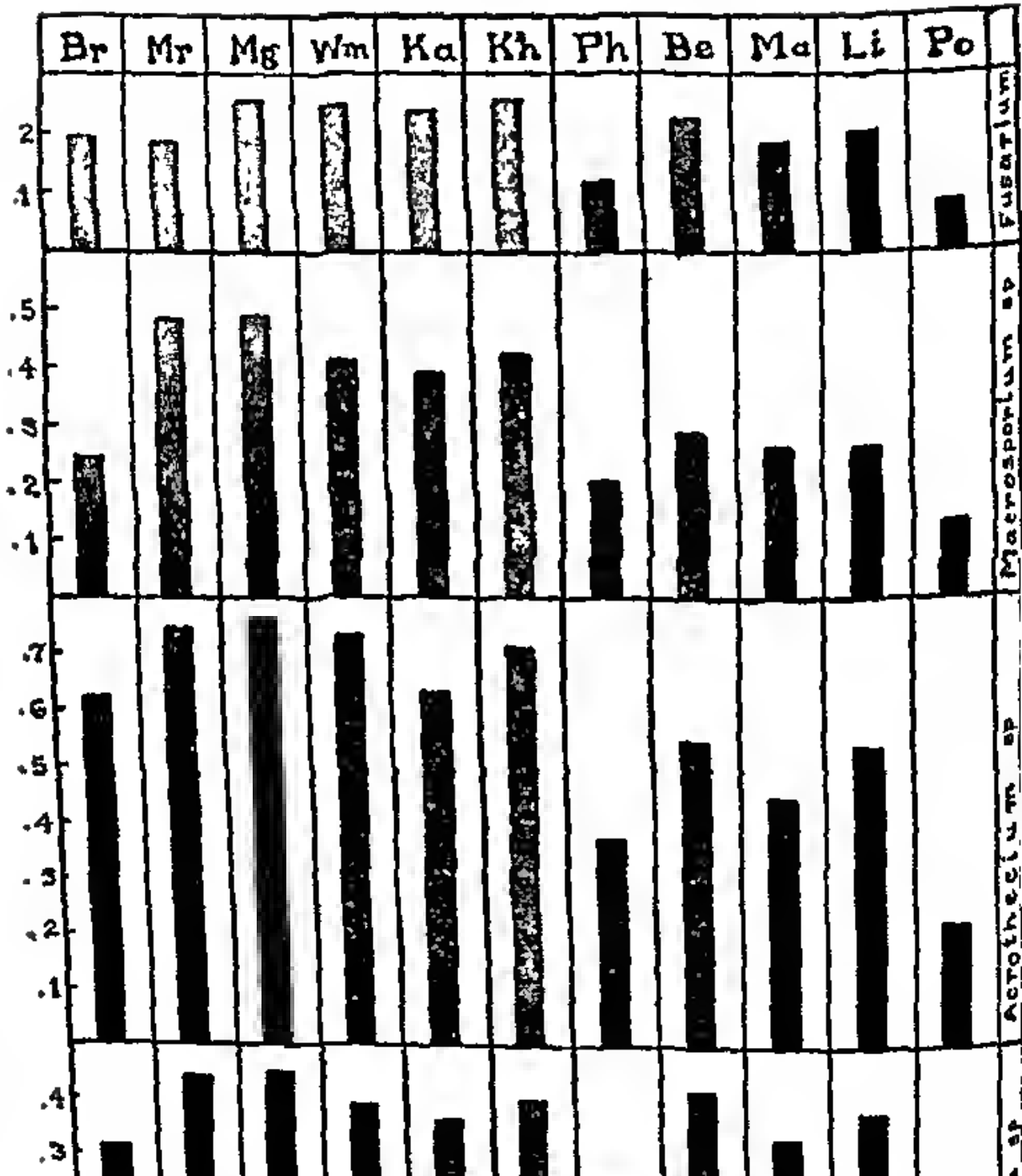
Text-Fig. 2. Graph showing the rate of linear growth of *Macrosporium* sp. on the various media.



Text-Fig 3 Graph showing the rate of linear growth of *Acrothecium sp* on the various media.



Text-Fig. 4. Graph showing the rate of linear growth of *Spicaria sp* on the various media.



Text-Fig 5 Graph showing the average rate of radial advance (indicated by th height of the figures from the base lines) of the four fungi on the various media.

The graph in the Text Fig. No. 3 shows that here also Green Mulberry agar proves to be the medium on which *Acrothecium* (No. 9) shows the greatest rate of spread. On Red Mulberry agar there is second highest rate of growth The series of the media showing decreasing growth rate in this fungus is Mg, Mr Wm, Kh, Ka, Br, Be, Li, Ma, Ph and Po. The same series is also obtained from the figures of the average rate of radial advance (see Text Fig 5).

In the graph in Text Fig No 4 we find that *Spicaria* (No. 10) also shows the greatest growth rate on Green Mulberry agar Second highest rate of spread is again shown on Red Mulberry agar The slowest rate of growth is found on Pomegranate agar The order showing a descending series of the rate of linear growth for the fungus is Mg, Mr, Be, Kh, Wm, Li, Ka, Ma, Br, Ph, and Po The same series also holds good for the average rate of radial advance on these media (see Text Fig. 5).

Macroscopic characters other than the rate of linear growth are tabulated below —

Fusarium incarnatum No 6.

Media	Growth	Aerial mycelium	Zonation	Colour	
				From above	From beneath.
Br	Moderate	Feeble	2, distinct.	Pale pink	Light Ochraceous buff.
Mr.	Very fair	Very feeble	Absent	Pale cinnamon pink	Darkish Walnut brown
Mg.	Good	Moderate.	Absent	Pale cinnamon pink	Near Ochraceous buff
Wm	Slight	Absent	Absent.	Pale Tellul buff.	Pale Cartridge buff
Ka	Scanty	Absent	Absent	Pale Tellul buff	Pale Olive buff.
Kh	Feeble	Absent.	Absent.	Pale Tellul buff	Pale Olive buff
Ph.	Very feeble	Absent	Absent	Pale Tellul buff.	Pale Olive buff.
Be	Very fair	Feeble	Absent	Pale cinnamon pink	Near dark Apricot orange
Ma.	Moderate	Very feeble.	Absent	Pale Tellul buff.	Pale Olive buff.
Li	Fair	Very feeble.	Absent.	Pale cinnamon pink	Pale Cartridge buff
Po.	Very feeble	Absent	Absent	Pale Tellul buff	Pale Olive buff

The aerial mycelium has a loose cottony texture where present On Mr, Li and Ma the aerial mycelium is almost absent but the colony is rather thick Then comes Kb, Wm, Ka, Ph and Po in order of the thickness of the colony. They are pellucid and have no aerial mycelium. On Brown's-starch only (Plate I, 1) there are two zones of better developed aerial mycelium. The media remain uncoloured but the colour as seen from beneath has been recorded.

Macrosporium sp No 7

Media	Growth.	Aerial mycelium.	Zonation	Colour	
				From above	From beneath.
Br	Fair	Fair	3, distinct.	Deep mouse grey	Deep neutral grey
Mr	Good	Very good	1, broad	Blackish mouse grey	Dusky purplish grey
Mg.	Vigorous.	Abundant.	1, indistinct.	Blackish mouse grey	Deep Bluish grey
Wm.	Moderate.	Fair	4, faint.	Dark neutral grey	Greyish slate.
Ka.	Moderate.	Feeble.	1, distinct.	Dark neutral grey	Pale greyish slate.
Kb.	Moderate.	Good.	3, distinct.	Deep mouse grey	Deep slate.
Ph.	Feeble	Feeble.	1, faint.	Dark neutral grey.	Pale slate.
Be.	Good	Very good	1, indistinct.	Dark mouse grey	Dark slate.
Ma.	Fair	Good	Absent.	Deep mouse grey	Greyish slate.
Li.	Fair	Good	Absent.	Dark mouse grey	Deep slate.
Po	Feeble.	Slight.	3, distinct.	Deep mouse grey	Slate.

In this fungus the deep mouse grey cottony mycelium is covered over by a whitish mycelium which is mostly present near the centre. On Mr, it forms a broad ring near the centre (Plate I, 5) and on Mg and Be it forms an indistinct ring at the same place The growing margin of the colony on Mr, Mg, Be, Li and Br had a Dark Ivy green colour. Unlike the previous one this fungus imparted colour to the substrata which appeared in a marked degree after about fifteen days' growth In Br the colour of the medium is present in a prominent ring 1 2 cms wide at a distance of about 2 cms from the centre.

Acrothecium sp. No. 9

Media	Growth	Aerial mycelium	Zonation	Colour	
				From above	From beneath
Br	Vigorous	Abundant	1, broad	Dark mouse grey	Slate grey
Mr	Very vigorous	Very abundant	3, faint	Dark mouse grey	Dark olive grey
Hg	Very vigorous	Very abundant	3, faint	Dark mouse grey	Green bluish slate
Wm	Vigorous	Abundant.	Absent	Dark mouse grey	Slate
Ka.	Very good	Very good	4, distinct	Dark mouse grey	Pale slate
Kh	Vigorous.	Abundant.	3, indistinct.	Dark mouse grey	Slate
Ph.	Good.	Good	Absent	Dark mouse grey	Slate grey
Be	Very vigorous	Very good	Absent.	Dark mouse grey	Blackish purple slate
Ma	Vigorous.	Very good	Absent	Dark mouse grey	Near castor grey
La.	Vigorous	Very good	Absent	Dark mouse grey.	Slate
Po	Fair	Fair	Absent	Dark mouse grey	Light grey

In *Acrothecium* the aerial mycelium has an wooly texture and dark mouse grey colour on all the media. On Br. there is a broad ring of white surface mycelium near the centre (Plate I, 12). On Bel agar there is no zonation but the culture shows a number of longitudinal grooves extending from the centre of the colony to the edge (Plate II, 3). The colour noted from beneath is the colour of the substrate which is developed to a marked degree after fifteen days' growth.

Spicaria sp No 10

Media	Growth	Aerial mycelium	Zonation	Colour	
				From above	From beneath
Br	Moderate	Fair	Absent	Pinkish white	Pinkish buff
Mr	Good	Fair	Absent	Pinkish white	Dark Walnut brown
Mg	Good	Good	Absent	Pinkish white	Light cinnamon buff
Wm	Feeble	Feeble	Absent	Pale pinkish white	Pinkish Cartridge buff
Ma	Feeble	Moderate	Absent	Pale pinkish white	Pale pinkish buff
Kb	Moderate	Feeble	Absent	Pinkish white	Pale pinkish buff
Ih	Very feeble	Feeble	Absent	Pale pinkish white	Pale pinkish buff
Be	Good	Good	Absent	Pinkish white	Dark Apricot orange
Mo	Moderate	Moderate	Absent	Pinkish white	Pale pinkish yellow
Li	Fair	Fair	Absent	Pinkish white	Pinkish buff
Po	Very feeble	Feeble	Absent	Pale pinkish white	Pale pinkish white

In this fungus the aerial mycelium is loose and cottony and has a pinkish white colour on all the media. On the last five media it is developed only in the centre of the colony The media remained uncoloured but the colour as seen from below has been recorded

(B)—Microscopic Characters

As advised by Brown and Horne (7) samples for comparative purposes were taken at about 1 cm distance from the centre when the cultures were fifteen days' old After noting the condition (vacuolation, etc.)

of the mycelium and the spores the slides were kept in glycerine and further examinations made

The shape of the spores remained practically constant and has been described before The mycelium and the spores of the fungi on all the media ranged from hyaline to vacuolate and in this respect no constancy was observed In no media were they granular.

Other characters are tabulated below—

Sporulation

Media	Fusarium	Macrosporium	Acrothecium	Spicaria
Br	Feeble	Very good	Very good	Good
Mr	Very good	Intense	Very good	Intense
Mg	Good	Very good	Intense	Intense
Wm	Feeble	Sparse	Fair	Fair
Ka	Feeble	Fair	Feeble	Fair
Kh	Fair	Good	Fair	Intense
Ph	Very sparse	Very feeble	Very feeble	Fair
Be	Good	Fair	Very fair	Very good
Ma	Fair	Feeble	Fair	Good
Li	Fair	Very fair	Good	Intense
Po	Very feeble	Sparse	Sparse	Fair

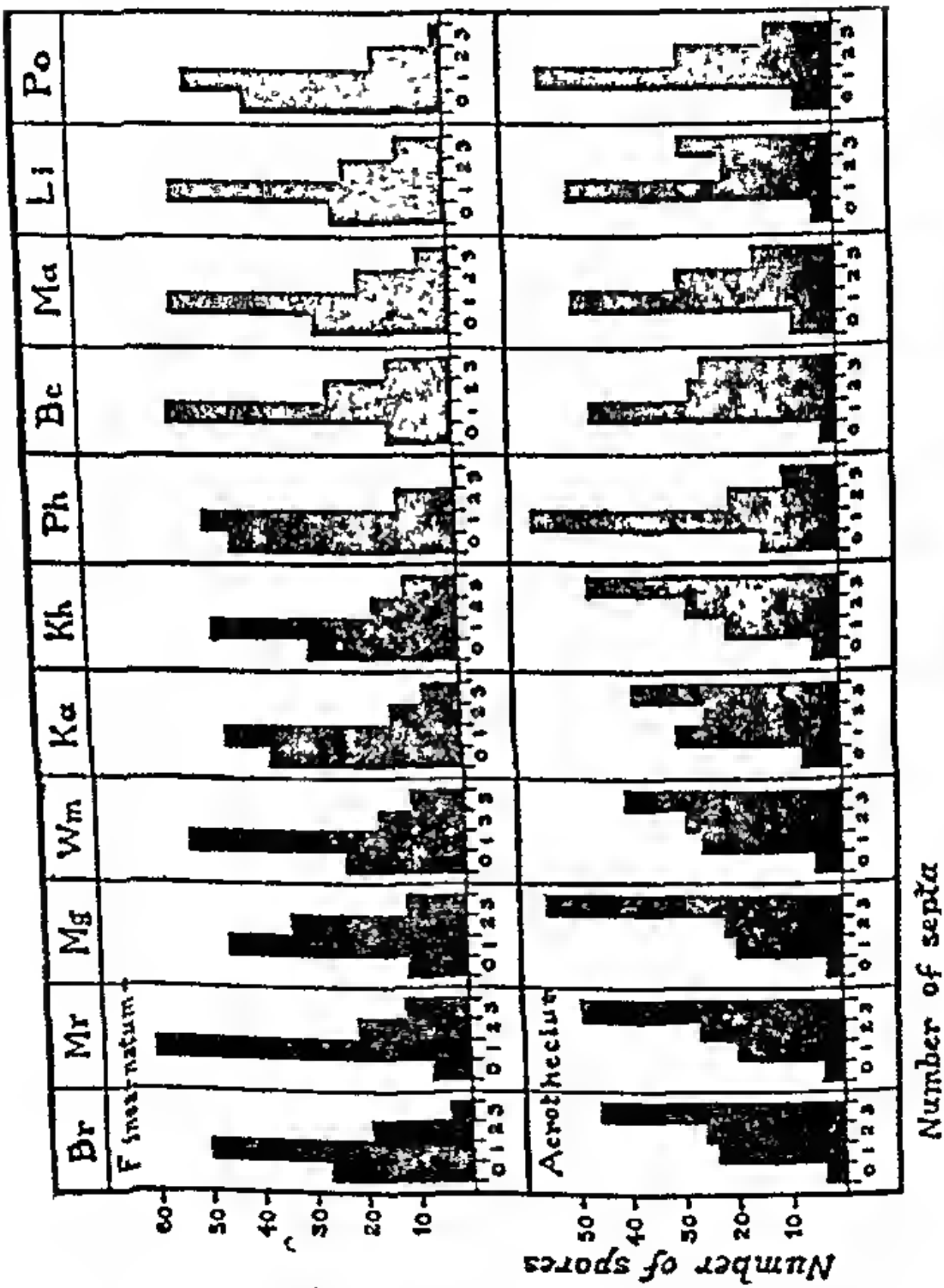
Septation —The spores were so counted as to avoid unconscious selection The results of 100 spore counts are shown graphically in Text Fig. 6, and the average septation is tabulated below

Average septation

Fungi	Br	Mr	Mg	Wm	Ka	Kh	Ph	Be	Ma	Li	Po
Fusarium	1 0	1 38	1 44	1 14	91	1 08	69	1 37	1 04	1 16	50
Acrothecium	2 14	2 21	2 2	2 04	2 15	1 94	1 20	1 74	1 51	1 72	1 45

Fungus No 7.—*Macrosporium sp.* The spores were muriform and no counting of septa was attempted.

Fungus No 10.—*Spicaria sp.* The spores were all a septate



From the Text Fig No. 6 it is seen that on all the media the septation mode of *F incarnatum* (No. 6) remains one, but on all of them, excepting Phalsa agar, 0- to 3-septate spores are found. There is greatest number of 1-septate and 3-septate spores on Red Mulberry agar but there is far greater number of 2-septate spores on Green Mulberry agar than on the former medium. So the average septation on the latter medium is higher If the average septation be the index of the suitability of the different media with regard to this character then the figures give us the following series in a decreasing order of suitability for this fungus Mg, Mr, Be, Li, Wm, Kh, Ma, Br, Ka, Po and Ph.

On the other hand *Acrothecium sp* (No. 9) shows a three mode septation on the first six media but on the last five media the septation mode is changed to one The largest number of 0-septate and 1-septate spores were again found on Phalsa agar which also showed the fewest 3-septate spores. So in the case of this fungus also there is lowest average septation on this medium. On Green Mulberry agar the number of the 3-septate spores and the average septation also are highest In this fungus the following series of media are obtained from the figures of the average septation The series in decreasing order ie, Mg, Mr, Ka, Br, Wm, Kh, Be, Li, Ma, Po and Ph.

Measurements —In *Fusarium incarnatum* (No. 6) the largest spores were obtained on Red Mulberry agar and Green Mulberry agar. The 0-septate spores were 9 5—13 8 × 3·5—4·3μ, average size 11 2 × 3 5μ The 1-septate spores were 14 7—20 7 × 3·5 – 5 2μ, average size 19 8 × 4 3μ. The 2-septate spores were 17 3—22·4 × 4·3—5·2μ, average size 20 7 × 5 2μ. The 3-septate spores measured 19 0—25·9 × 4 3—5 2μ, average size 22·4 × 5 2μ. On Wm, Kh, Be, Ma and Li the next largest speres were obtained The average size of the 3-septate spores were 19 8 × 5 2μ On Br, Po and Ph spores of the lowest dimensions were found. The 0-septate

spores ranged from 7·8—11·2×3·5—4·3μ. The 3-septate spores were 17·3—22·4×3·5—5·2μ, average size—18·2×5·2μ

Macrosporium (No 7) also shows the largest spores on Red Mulberry and Green Mulberry agars The measurements are 10·4—62·1×6·9—16·4μ On the rest of the media the spores generally varied from 10·4—51·8×6·9—15·5μ.

In *Acrothecium* sp. (No. 9) greatest dimensions of the spores are found on the following media—Mr, Mg, Kh, Bo, Ma and Li The spores generally measured, 0-septate ones 6·9—14·7×6·9—8·6μ, average size—13·8×6·9μ. 1-septate spores 10·4—17·3×6·9—8·6μ, average size—15·5×6·9μ 2-septate spores 13·8—19·0×6·9—10·4μ, average size—16·4×8·6μ. 3-septate spores 17·3—25·9×8·6—10·4μ, average size—20·7×10·4μ. On the rest of the media, viz., Br, Wm, Ka, Po and Ph, the spores were shorter and the 3-septate spores measured 15·5—20·7×7·8—9·5μ, average size—18·2×8·6μ.

Spicaria sp (No. 10) shows largest spores on Red Mulberry agar, the measurements being 6·9—22·4×3·5—6·0μ, average size—13·8×5·2μ. On Mg, Kh, and Bo next higher measurements are found—the average size of the spores being 13·0×5·2μ On Br, Ma, and Li, the average size of the spores is 11·2×3·5μ On the rest of the media, viz., Wm, Ka, Po and Ph the shortest spores are observed They measured 5·2—7·3×2·6—4·3μ, average size—10·4×3·5μ.

True chlamydospores were found in *Acrothecium* (Plate III, 4) They were absent on Brown's-starch but on other media they were present in fair numbers Some swellings of the hyphae with an thick wall were however found in almost all the cultures of *Fusarium incarnatum* (Plate III, 8) It is not certain whether they are mere swollen hyphae or are of the nature of chlamydospores.

Saltations.—1. No. 6a—This saltant of *F. incarnatum* arose as two sectors on Red Mulberry agar (Plate I, 2)

It had better developed white aerial mycelium than the parent and probably had a faster growth rate also, because the sectors outgrew the radius of the parent colony. Medium remained uncoloured Sporulation was very good and the spores were hyaline to vacuolate. The septation mode was 1 and the average septation was 1 30 The measurements were the same as that of the parent

2. No 6b.—This saltant (Plate I, 3) of *F. incarnatum* arose on Green Mulberry agar and occupied nearly half of the diameter of the colony It was very etaling and the colony never reached the edge of the plate. The margin of the colony was wavy. There was much less aerial mycelium than the parent The medium below it remained uncoloured Sporulation was good, the septation mode was one and the average septation was 1·30 The measurement of the spores were the same as that of the parent

3. No. 9a —This saltant of *Acrothecium* (Plate I, 12) arose in the form of sectors on two of the three plates of Brown's starch It had only a very moderate amount of aerial mycelium. The colour of the aerial mycelium was Vinaceous russet (reddish) and in this respect differed very markedly from that of the parent which was dark mouse grey (blackish). Colour of the medium below was light Russet Vinaceous. The red colour of the saltant seemed to be present on the cell wall only. Sporulation was very meagre The spore mode was 3 and the average septation was 1 96. The spores were shorter than the parent, their measurements being 3-septate spores 13 8—18·2 × 6 9—9·5 μ, average size—15·5 × 8 6 μ

4 No 10a —This saltant of *Spicaria* (Plate II, 5) arose on Red Mulberry agar and occupied the major part of the culture so that the parent assumed the form of a sector. It had less of aerial mycelium and the colour below was Russet Sporulation was intense and the spores measured the same as that of the parent.

COMPARISON OF RESULTS AND DISCUSSION

From the observations presented before it is seen that almost all the characters of the four fungi show the greatest development on Green Mulberry agar The only exceptions are found in sporulation where *Fusarium* and *Macrosporium* show slightly better sporulation on Red Mulberry agar The measurement of the spores of *Spicaria* is also slightly greater on the latter medium. Zonation, however, is rather poor on Green Mulberry agar On Red Mulberry agar there is generally second best development of all the characters, though sometimes, as in the case of the development of aerial mycelium in *Fusarium* and *Spicaria* and in the sporulation of *Acrothecium*, it is superseded by other media Only the linear growth rate of *Fusarium* is rather slow on this medium

All the fungi in a like manner show very feeble development on Phalsa agar and Pomegranate agar There is least rate of growth of all the fungi on Pomegranate agar and the colour and development of the aerial mycelium is the feeblest In these respects Phalsa agar is only slightly better than it But as regards sporulation, septation and measurements of the spores the order is reversed. Phalsa agar proves to be the worst medium and Pomegranate agar is only slightly better

The rest of the media vary in their positions among the other media with regard to the different characters On Bel agar there is very good development of most of the characters and often it equals Red Mulberry agar or even Green Mulberry agar But the linear growth rate of *Fusarium*, *Macrosporium* and *Acrothecium* on this medium is slow In almost every case Luchi agar comes next to Bel agar but fungi Nos 7, 9 and 10 show slightly better sporulation on the former medium than on the latter Not a very good development is shown by the fungi on Mango agar. On this medium

their rate of growth is very slow but other characters show a fair development With these the standard synthetic medium Brown's starch does not compare very favourably All the fungi show a slow rate of growth on this medium but some characters do show a good development on it. Thus in sporulation of *Macrosporium and Acrothecium* it approaches Green Mulberry agar and Red Mulberry agar respectively The aerial mycelium of these two fungi shows a fair development on this medium On Kharbuja agar there is very rapid rate of growth but excepting sporulation other characters fail to show a good development on it Similarly, on Water-melon agar and Kakri agar the fungi show a fast growth rate but all the other characters are developed poorly. Zonation, however, is better shown on Kakri agar than on any other media

It would be very difficult, if not impossible, to account for all these behaviours of the fungi on the various media It would be hazardous to assign the results to any particular factor in these multi-conditioned metabolic processes This is more particularly so in the event of an almost entire absence of knowledge about the composition of the fruit juices which have been employed However, the following conclusions seem probable

It has been found that on Green Mulberry agar there is highest rate of spread and best development of other characters but on Water-melon agar and Kakri agar a high growth rate is found associated with a feeble development. Thus it is seen that a greater rate of linear growth does not always correspond to a greater development with regard to other characters This has also been noticed by Lacy (11) working with the same four fungi Similar conclusions have also been reached by other workers as Brown (6) and Stevens and Hall (15). The latter state that "no correlation is noted between the rapidity of linear growth and the nutritive value of the medium. In many cases most rapid

linear growth occurred in what was surely the poores medium" It has already been pointed out that this worl was carried out at a rather high temperature According to Balls (1) there is an optimum temperature for growth beyond which the growth curves decrease and Mitra (12 states that for a given fungus the optimum temperature fo growth varies with the medium He finds that Brown' medium gives in general a lower optimum temperatur for growth than other media as Prune juice agar, etc For these fungi also it is possible that the temperature a which they were grown were rather higher above th optimum more especially for Brown's-starch but in th absence of any definite knowledge it is best not to infe any conclusion. Acidity of the medium has a marke effect on the growth of Fungi Working on *Fusarium* Horne and Mitter (10) obtained curves of the usual optimu type and found that some strains were more tolerant c acid than others According to Boyle (3) the pH-limi and optimum for growth of *Fusarium* depend on th medium The results obtained in this work probabl justify these conclusions Both Red Mulberry agar an Phalsa agar have a high concentration of acid but on th former medium there is much better development of a the fungi than on the latter. On the other hand, bett development is obtained on the less acidic Green Mulber agar than on the more acidic Red Mulberry agar. Zonatic has been, from time to time, attributed to various caus agencies Bisby (2) attributes this character to alternati light and darkness and according to Mitra (12) this effe is more clearly marked in the neighbourhood of the optimu temperature Hedgcock (9) finds that in *Cephalotheciu* daily variation in temperature is not the cause of zonati and according to Brown (6) this character is a function of particular strain and has some systematic value In th work it is noted that though exposed to the same conditio

a fungus formed zones on some medium and not on others. Thus here it is seen that zones are produced by a particular strain on a particular medium under certain conditions and that no general conclusion can be drawn. Lastly comes the question of saltations As has been noted by others here also it has been found that such characters as the development and colour of the aerial mycelium, sporulation and size of the spores, etc, may show a marked change in these sudden variants. The shape of the spores, however, remained the same though in saltants this character even has been observed by Mitter (13) to vary. As such characters are used in the determination of the species of the genera it is important to exercise great care in doing so.

From the results obtained in this work the author recommends the media in the following decreasing order of suitability for the cultivation of fungi, Green Mulbarry agar, Red Mulberry agar, Bel agar, Lichi agar, Mango agar, Brown's-starch, Kharbuja agar, Water-melon agar, Kakri agar, Pomegranate agar and Phalsa agar.

In conclusion the writer acknowledges his indebtedness to Prof J. H Mitter for the suggestion of the problem and guidance and also to Mr R N. Tandon for his help and interest in the investigation

SUMMARY

1 The effect of eleven media prepared from the juices of fresh fruits on the growth of four fungi, namely, species of *Fusarium Macrosporium*, *Acrothecium* and *Spicaria* has been studied

2 Best development of almost all the characters of all the four fungi is found on Green Mulberry agar and generally the second best development is found on Red Mulberry agar

3 On the other hand, most feeble development as regards all the characters is shown on Pomegranate agar and Phalsa agar by all the four fungi. The other media occupy various positions in the series with regard to the development of various characters The standard synthetic medium, Brown's-starch, is much less favourable to the growth of these fungi when compared to many of the media employed.

4. On certain other media as Kharbuja agar, Water-melon agar and Kakri agar a fast rate of linear spread is found associated with a poor development of other characters It is inferred that the rate of linear growth may not give an indication of the amount of growth or the suitability of the medium This is in agreement with the conclusions reached by other workers

5. Zonation is found to be produced by a particular strain on a particular medium under certain conditions and no general conclusions could be based as regards the formation of zones either with the individual fungi or the media.

6 The nature of the medium is found to be more effective than its pH-value.

7 Saltations occurred in *Fusarium* on Red Mulberry and Green Mulberry agars, in *Acrothecium* on Brown s-starch and in *Spicaria* on Red Mulberry agar. Such characters as the development and colour of the aerial mycelium, sporulation and size of the spores are found to be markedly different from those of the parents

8. From the results obtained the author recommends the media in the following decreasing order of suitability for the cultivation of Fungi—Green Mulberry agar, Red Mulberry agar, Bel agar, Lichi agar, Mango agar, Brown s-starch, Kharbuja agar, Water-melon agar, Kakri agar, Pomegranate agar and Phalsa agar.

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EXPLANATION OF PLATES I, II and III

Illustrating A. K. Mitra's Paper on Comparative Values of Fruit Juice Media.

Plate I

Fig. 1. F incarnatum on Brown's-starch Culture 11 days old.
Fig 2 F incarnatum Parent and Saltant on Red Mulberry agar Culture 13 days old.
Fig 3 F incarnatum Parent and Saltant on Green Mulberry agar. Culture 20 days old.
Fig 4 Macrosporium on Brown's-starch Culture 11 days old
Fig. 5 Macrosporium on Red Mulberry agar Culture 11 days old
Fig. 6 Macrosporium on Green Mulberry agar Culture 11 days old
Fig. 7. Macrosporium on Water-melon agar Culture 11 days old
Fig 8. Macrosporium on Kakri agar. Culture 11 days old.
Fig 9 Macrosporium on Kharbuja agar Culture 11 days old.
Fig. 10 Macrosporium on Bel agar. Culture 13 days old
Fig. 11 Macrosporium on Lichi agar. Culture 10 days old
Fig 12. Acrothecium on Brown's-starch, Parent and Saltant Culture 10 days old

Plate II

Fig 1 Acrothecium on Kakri agar Culture 5 days old
Fig 2. Acrothecium on Kharbuja agar. Culture 10 days old.
Fig 3 Acrothecium on Bel agar Culture 10 days old
Fig 4 Spicaria on Brown's-starch Culture 11 days old
Fig 5 Spicaria on Red Mulberry agar. Parent and Saltant Culture 10 days old
Fig 6 Spicaria on Green Mulberry agar. Culture 10 days old.
Fig 7 Spicaria on Kakri agar. Culture 10 days old
Fig 8 Spicaria on Bel agar Culture 20 days old
Fig 9 Spicaria on Mango agar Culture 20 days old.
Fig. 10 Spicaria on Lichi agar. Culture 20 days old.
Fig 11 Spicaria on Phalsa agar. Culture 20 days old.
Fig 12. Spicaria on Pomegranate agar Culture 20 days old

Plate I

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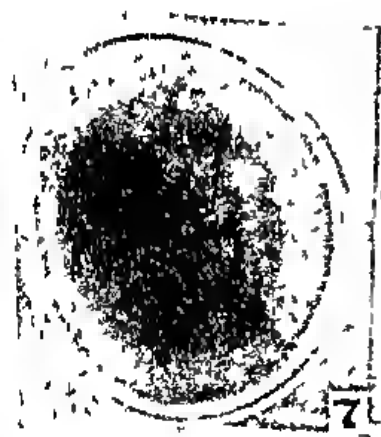

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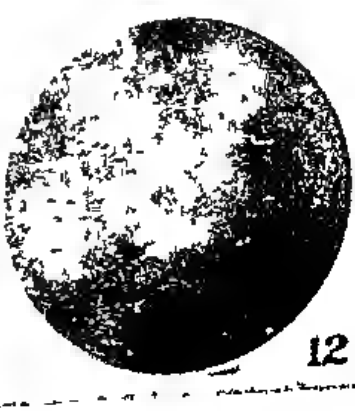

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Plate II


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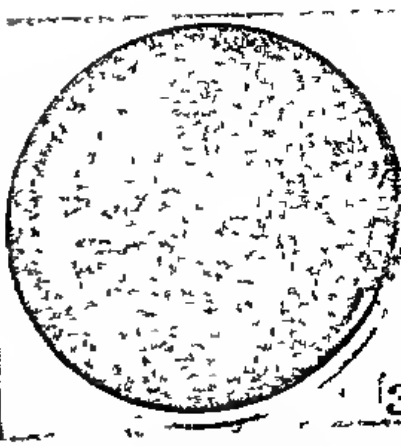
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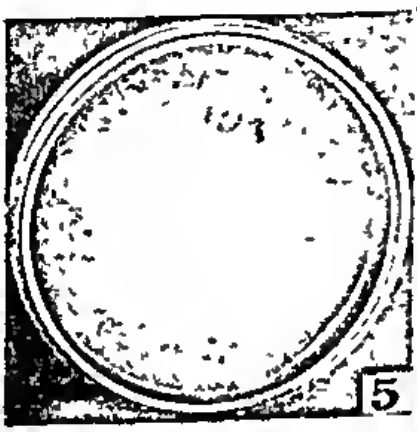
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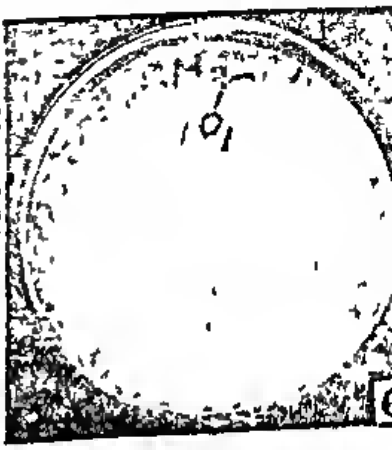
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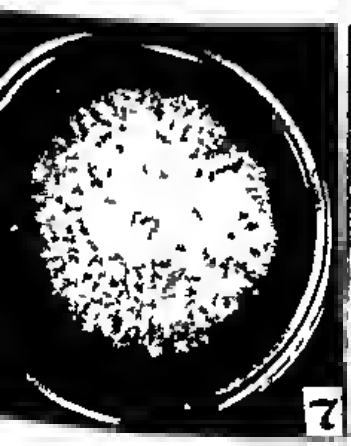
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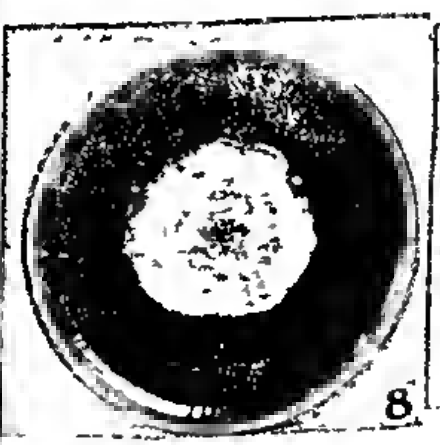
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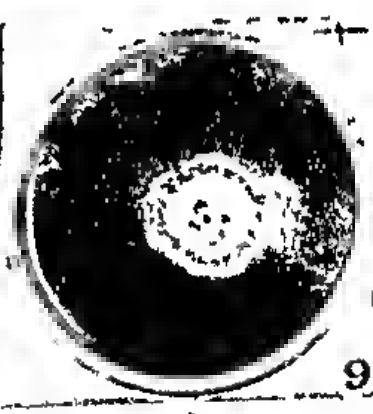
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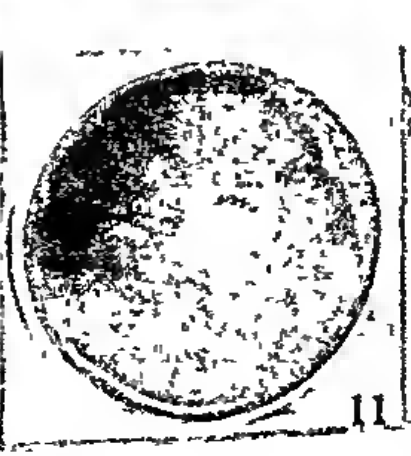
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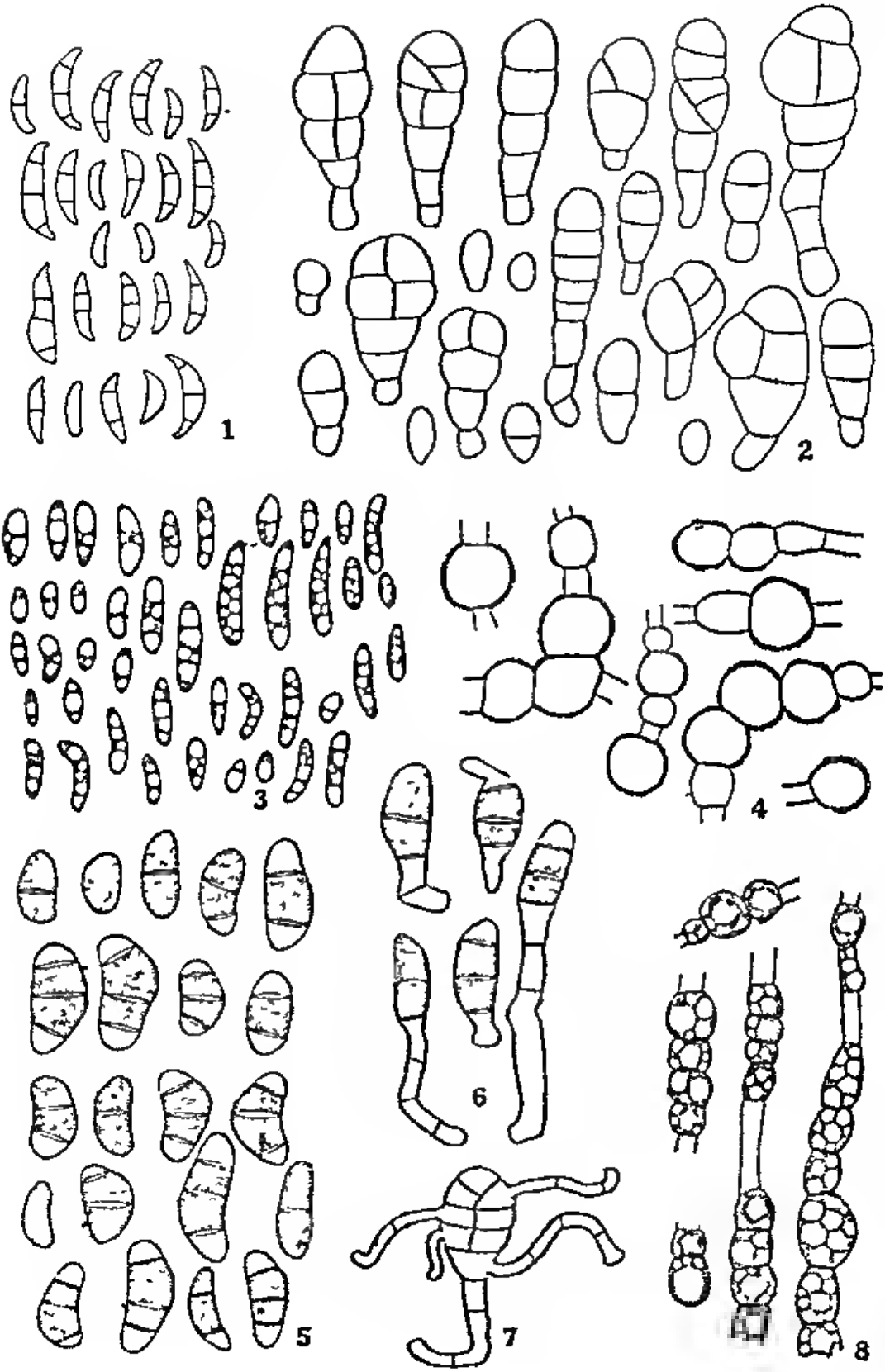
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Plate III

(All the figures were originally drawn at a magnification of about X1600 with the aid of a Camera Lucida and have been reduced to about X$\frac{1}{2}$)

Fig 1 Fusarium incarnatum Spores from Various Media
Fig. 2 Macrosporium Spores from Various Media
Fig. 3. Spicaria. Spores from Various Media.
Fig 4 Acrothecium. Chlamydospores from Various Media
Fig 5 Acrothecium Spores from Various Media
Fig 6 Acrothecium. Germination of Spores
Fig 7. Macrosporium Germination of a Spore
Fig 8 Fusarium incarnatum. Swollen Hyphae on Various Media

SECTION IV

PHYSICS

ON A NEW KIND OF CHARACTERISTIC X-RAYS

BY

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For some time past, the writer of the present note has been thinking of the possibility of having a new kind of characteristic X-rays which stand in the same relation to the usual K and L-spectra as complex optical spectra of elements stand to optical alkali spectra. The K and L-spectra and other usual characteristic X-ray spectra are due to the removal of *one* electron from any closed shell, and the subsequent filling up of this shell by an electron from some external shell according to the rules of quantum mechanics The alkali-like structure of X-ray spectra is due to the operation of the Pauli principle, according to which *defect* of an electron from a closed shell gives rise to the same spectroscopic terms as excess of one electron outside a closed shell. Is it not just possible that in the act of bombardment by the cathode rays, more than one electron is displaced from one or more shells simultaneously? As such phenomena are quite common in the excitation of optical spectra by cathode ray bombardment, e.g., in Franck and Hertz's experiments, we can expect the same to hold good when we bombard the interior of the atom with electrons of sufficient energy. Supposing now in one act of bombardment, both electrons in the K-shell are carried off, what will happen next? As this state is unstable, two electrons will now jump from the L, or higher shell, and fill up the K-level It can be shown from principles of quantum mechanics that one of these transitions will be allowed, the other disallowed. But the frequency of the line or lines emitted will be approximately

$(K_1 - L_1) + (K_1 - L_2)$ and hence it will have *approximately* double the energy of ordinary K-radiation Exact calculation shows that there will be two regular lines $^1S_0 - ^1P_1$, $^1S_0 - ^3P_1$ and there may be besides two forbidden lines, $^1S_0 - ^3P_0$, $^1S_0 - ^3P_2$ There may be another group corresponding to K_2, and an intermediate group corresponding to $K_1\ K_1 \longleftarrow L_1\ M_2$ or $K_1\ K_1 \longleftarrow L_2\ M_1$

It is well known that several lines of obscure origin appear on the shorter side of K lines and are known as spark lines. It appeared to me from scrutiny of existing literature that two at least of these spark lines α_3, α_4 are in reality the double transition lines obtained in the second order Acting on this hypothesis I directed my colleague Prof Bhargava and my scholar, Mr J. B Mukerjee to try to get these lines We have to expose our plates at approximately half the wavelength of copper $K\alpha_3$ radiation, and maintain the voltage at a steady value of 40000 which is about double the excitation voltage of $K\alpha$-line of copper When we developed the plate after twenty hours' exposure a sharp line was found at the expected position impressed on a faint continuous background Rough measurements showed that it had a wavelength of λ 760 X units while the value of λ for $K\alpha$ of copper is 1530 X units. The measured wavelength is approximately half the expected value, but the measurements were rough, and as the method of fixed crystals was used, there may be large errors in the measurement of the angle. Theoretical considerations show that owing to coupling phenomena the wavelengths of $^1S_0 - ^1P_1$ and $^1S_0 - ^3P$ lines may considerably deviate from the half-value of $K\alpha$ or $K\beta$. The hypothesis of double ionisation and double transition thereby receives good confirmation. About a month previous to this work, working in collaboration with Prof. Bhargava, and Mr. Mukerjee, evidence of double transition L-spectrum of tungsten was obtained.

But we postponed announcement of the result till further confirmation was received

I have no doubt that the phenomenon is general, and before long, workers in this field will be looking for double transition K and L-spectra of all elements. This survey will take much time and labour, but when it is fairly done, there is no doubt that the results will throw a flood of light on the structure of the atom